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ANALYTICAL TECHNIQUES FOR THE NATIONAL
EUTROPHICATION RESEARCH PROGRAM

Federal Water Pollution Control Administration
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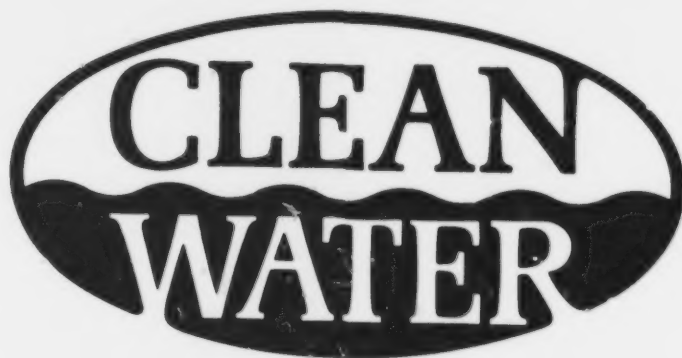
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FEDERAL WATER POLLUTION CONTROL ADMINISTRATION



ANALYTICAL TECHNIQUES

for

THE NATIONAL EUTROPHICATION RESEARCH PROGRAM

June 1969

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U S Department of Commerce
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Participants in the
National Eutrophication Research Program

February 28, 1969

Chief, National Eutrophication Research Program

Analytical Techniques

The FWPCA research activities in eutrophication control include intramural and extramural efforts. Intramural efforts at the Pacific Northwest Water Laboratory, the Ely, Minnesota, field site, and elsewhere relate to mechanical, chemical, biological, and ecological control processes. Extramural efforts in the same areas consist of some 20 projects ranging broadly in their specific objectives. This grant- and contract-supported work involves a scientific work staff of some 80 persons. Because of the magnitude of these efforts and the variability of analytical techniques now used, research results are not always acceptably comparable. This compilation of appropriate analytical techniques, therefore, has been prepared as a step toward assuring that all participants in the National Eutrophication Research Program are using the same procedures.

These analytical techniques are consistent with FWPCA Official Interim Methods for Chemical Analysis of Surface Waters, September 1968. They also are the ones now in use in the intramural activities of the National Eutrophication Research Program. It is intended that these procedures be used hereafter in all eutrophication research activities supported by FWPCA, and they have been designed and assembled to meet this objective.

This manual was prepared by Daniel F. Krawczyk, Chief of the Consolidated Laboratory Services Program, with the help of many staff members of the Consolidated Laboratory Services Program and the National Eutrophication Research Program.

A. F. Bartsch

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Nickel

Potassium

Sodium

Tungsten

Vanadium

Zinc

Arsenic

123

Boron

128

Chlorophyll

132

pH

1. Scope

1.1 This method covers the electrometric measurement of the pH of water by means of a glass electrode. The term pH is defined in terms of E, the electromotive force between glass and a reference electrode when immersed in an aqueous solution in volts, and E_s , the electromotive force obtained when the electrodes are immersed in a reference buffer solution in volts as follows:

$$pH = pH_s + \frac{(E - E_s)F}{2.3026RT}$$

where

pH_s = of reference buffer

F = Faraday

R = gas constant

T = absolute temperature

2. Sampling

2.1 It would be desirable to insert the electrodes directly into an aqueous system. If samples must be collected, use glass or plastic containers that will not release the constituents which affect the pH determination.

3. Preservation

3.1 Analyze samples as rapidly after collection as possible. There are no known preservatives that will stabilize the pH condition of the sample.

4. Apparatus

4.1 A pH meter with associated glass and reference electrodes. The pH meter shall have a potentiometric measuring circuit with a null

indicator or a measuring circuit calibrated for direct deflection, preferably with temperature compensation.

4.2 Reference electrode calomel.

4.3 Indicating electrode, glass electrode.

4.4 Stirrer, mechanical, preferably of magnetic type.

5. Reagents

5.1 Reference buffer solution pH 7 purchased commercially or prepared from texts on buffer preparation.

5.2 Reference buffer solution pH 4 purchased commercially or prepared from texts on buffer preparation.

5.3 Reference buffer solution pH 10 purchased commercially or prepared from texts on buffer preparation.

6. Procedure

6.1 Turn on the instrument, and allow it to come to electrical balance as per manufacturer's instructions. Thoroughly rinse the electrodes wiping excess moisture with fine paper towelling. Immerse electrodes in reference buffer, turn on stirrer for gentle agitation (use buffer in the same range as pH of expected samples), and note temperature. Make any temperature adjustment as per manufacturer's instruction.

6.2 Remove electrodes from the buffer and thoroughly rinse electrodes, wiping excess moisture with fine paper towelling. Immerse the electrodes into the test samples, turn on the stirrer for agitation, note temperature, make any adjustment for temperature, and read or dial the pH value.

6.3 Check against the buffer after every fifth sample and at the conclusion of a group of samples.

7. Calculation

7.1 Report pH directly and temperature of pH reading.

REFERENCES

Feldman, I. Use and abuse of pH measurements. *Analytical Chemistry*, 28(12):1859-1866. 1956.

Laitinen, H. A. *Chemical Analysis*. McGraw-Hill, New York, 1960.
p. 5-55.

ALKALINITY

1. Scope

1.1 The alkalinity determination measures the hydroxyl carbonate and bicarbonate ions present in water. The addition of acid may dissociate solutes which will react with the acid to give an indication of alkalinity. The method is used to develop an electrometric titration curve. The equivalence point (end point) is determined by plotting pH as the ordinate and acid (in ml) as the abscissa.

2. Sampling

2.1 The samples can be collected in plastic or glass bottles that have been thoroughly rinsed in distilled water before they are dried.

3. Preservation

3.1 Samples should be analyzed as soon after collection as possible.

4. Apparatus

4.1 Electrometric pH Measurement Apparatus, conforming to the requirements given in the Methods of Test for pH.

4.2 Burets, 50 ml, graduated in 0.1 ml divisions.

5. Reagents

5.1 Prepare .2N H_2SO_4 acid titrant by diluting 5.6 ml of concentrated sulfuric acid to a liter.

5.2 Prepare .02N H_2SO_4 . Withdraw 100 ml of .2N and dilute to a liter. Standardize electrometrically against sodium carbonate (Na_2CO_3).

5.3 Prepare by heating sodium bicarbonate (NaHCO_3) for 2 hours at 270°C . The inflection point corresponding to the complete titration of the carbonic acid salts will be very close to pH 3.9.

6. Procedure

6.1 Mount the glass and reference electrodes in two of the holes of a clean three-hole rubber stopper chosen to fit a 300 ml, tall form, Berzelius beaker without a spout. Place the electrodes in the beaker. Rinse the electrodes first with water, then with a portion of the sample. Following the final rinse, drain the beaker completely.

6.2 Pipet a 100 ml aliquot of the sample into the beaker through the third hole in the stopper. Hold the tip of the pipet near the bottom of the beaker while discharging the sample.

6.3 Measure the pH of the sample in accordance with the Method of Test for pH.

6.4 Add the applicable 0.02N standard solution in increments of 0.5 ml or less, mixing the solution thoroughly. Determine the pH when the mixture has reached equilibrium as indicated by a constant pH reading. Mechanical stirring, preferably of the magnetic type, is required for this operation. Mixing by means of a gas stream is not permitted. Continue the titration until the necessary data have been obtained. If the titration requires appreciably more than 25 ml of standard solution, use a 0.1N solution prepared and standardized in the same manner.

6.5 To develop a titration curve, plot the cumulative milliliters of standard solution added to the sample aliquot against the observed pH values.

7. Calculations

7.1 Alkalinity in the vicinity of pH 8.3 is calculated as follows:

Find the center of the steeply descending portion of the curve and chop a vertical line to the abscissa to find the amount of acid (in ml) required to reach the end point.

Phenolphthalein Carbonate bicarbonate Alkalinity as CaCO_3 =
 or
 Carbonate hydroxide
 or
 Hydroxide

$$\frac{(\text{ml stand. acid}) \times (\text{N of acid}) \times 50,000}{\text{ml of sample}}$$

7.2 Alkalinity in the vicinity of pH 4.5 - 4.0 is calculated as follows: Find the center of the steeply descending portion of the curve and drop a vertical line to the abscissa to find the amount of acid (in ml) required to reach the end point.

Total alkalinity CaCO_3 = $\frac{(\text{total ml acid} \times (\text{N of acid}) \times 50,000)}{\text{ml of sample}}$
 or
 T

where N = Normality of standard acid solution

7.3 Alkalinity Relationships

If	Then alkalinity present as		
	OH	CO_3	HCO_3
P=0	0	0	T
P 1/2T	0	2P	T-2P
P=1/2T	0	2P	0
P 1/2T	2P-T	2(T-P)	0
P=T	T	0	0

Key: P - phenolphthalein alkalinity
 T - total alkalinity

7.4 Example calculation: ml sample = 100; ml titrant at equivalence point = 3.2; N of acid = 0.019.

$$\text{Total alkalinity} = \frac{(3.2) (0.019) (50,000)}{100} = 30 \text{ mg/l as CaCO}_3$$

REFERENCES

Dong, G., Giusti, R. L., and Greenberg, A. E. How to make alkalinity measurements in water. *Water and Sewage Works*, 104(11):509-511. 1957.

Gast, J. A., and Thompson, T. G. Determination of alkalinity and borate concentration of sea water. *Analytical Chemistry*, 30(9):1549-1551. 1958.

Laitinen, H. A. *Chemical Analysis*. McGraw-Hill, New York, 1960. p. 5-56.

Larson, T. E., and Henley, L. M. Determination of low alkalinity or acidity in water. *Analytical Chemistry*, 27(5):851-852. 1955.

TOTAL ORGANIC CARBON, SOLUBLE ORGANIC CARBON, TOTAL CARBON

1. Scope

1.1 The total carbon, organic and inorganic, is measured by injection of a water sample into a high temperature combustion tube containing a catalyst. The carbon present as CO_2 is measured as the CO_2 gas. Carbon present in organic forms is oxidized to CO_2 and measured as CO_2 .

1.2 To determine total organic carbon the sample is acidified with a mineral acid to pH 2-3 and purged with nitrogen.

1.3 Soluble organic carbon is determined on a sample which has been filtered through a well rinsed glass fiber filter, acidified with a mineral acid, and purged with nitrogen.

2. Sampling

2.1 Samples should be collected in glass containers or in plastic containers that do not leach organics on contact with water.

3. Preservation

3.1 For total organic carbon and soluble organic carbon samples can be preserved with H_2SO_4 in quantities to reduce pH to 2-3 and kept refrigerated in tightly stoppered bottles until the start of analysis.

3.2 Total carbon analysis should be performed immediately on receipt of sample since organic components in aqueous solutions are subject to oxidation or bacterial decomposition.

4. Interferences

4.1 Saline and brackish water deteriorate the combustion tube.

5. Apparatus

- 5.1 Combustion tube.
- 5.2 Flowmeters, pressure regulators.
- 5.3 Blender.
- 5.4 Sample injection device.
- 5.5 Water trap.
- 5.6 CO₂ measuring device.
- 5.7 Recorder.

6. Reagents

- 6.1 Nitrogen gas.
- 6.2 H₂SO₄ - H₃PO₄, conc.
- 6.3 Primary organic standard sodium oxalate.
- 6.4 Primary inorganic standard calcium carbonate.

7. Procedure

- 7.1 Follow manufacturer's instruction in use of proprietary devices.
- 7.2 If water or wastewater samples contain an appreciable amount of suspended solids and or fibers, the samples must be thoroughly homogenized.
- 7.3 For total carbon, inject the sample through the inject device. Make two other similar injections to determine the peak height of sample.
- 7.4 For total organic carbon and soluble organic carbon, acidify sample with conc. H₂SO₄ to pH of 2-3 and purge sample for three minutes with nitrogen.

- 7.4.1 Inject sample into sample injection device.

7.5 Run a set of standards and a blank each day when analyzing for carbon. While running samples, run a standard at least every hour.

7.5.1 When analyzing for soluble organic carbon, a filtered blank must be run to determine contribution from filter.

8. Calculation

8.1 Calculation is carried out as follows:

Mg/l carbon =

$$\frac{(\text{sample peak hgt.} - \text{blank peak hgt.}) (\text{standard curve slope})}{\text{sample size}}$$

REFERENCES

Schoffer, R. B., et al. Application of carbon analyzer in waste treatment. Journal Water Pollution Control Federation, 37(11):1545-1566. 1965.

Van Hall, C. E., Safranko, J., and Stenger, V. A. Rapid combustion method for the determination of organic substances in aqueous solution. Analytical Chemistry, 35(3):315-319. 1963.

Van Hall, C. E., and Stenger, V. A. An instrumental method for rapid determination of carbonate and total carbon in solutions. Analytical Chemistry, 39(4):503-507. 1967

DISSOLVED OXYGEN

Azide Modification of Iodometric Method

Full Bottle Technique

1. Scope

1.1 This method is applicable for use with most wastewaters and streams that contain nitrite nitrogen and more than 1 mg/l of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 ml fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/l ferric iron.

2. Sampling

2.1 Where possible, collect the sample in a 300 ml BOD incubation bottle. Special cautions are required to avoid entrainment or solution of atmospheric oxygen or dissolution of dissolved oxygen.

2.2 Where samples are collected from shallow depths (less than 5 feet) use of an APHA-type sampler is recommended. Use of a Kemmerer-type sampler is recommended for samples collected from depths of greater than 5 feet.

2.3 When a Kemmerer is used, the BOD sample bottle should be filled to overflowing. (Overflow for approximately 10 seconds.) Outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling bottle.

2.4 The sample temperature should be recorded at time of sampling as precisely as required.

3. Preservation

3.1 Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron. If samples must be preserved either 3.1.1 or 3.1.2 below may be employed.

3.1.1 Add 2 ml of manganous sulfate reagent and then 2 ml of alkali azide reagent to the sample contained in the BOD bottle. Both reagents must be added well below the surface of the liquid. Stopper the bottle immediately and mix the contents thoroughly. The sample should be stored at the temperature of the collection water, or water sealed and kept at a temperature of 10 - 20°C, in the dark.

3.1.2 Add 0.7 ml of concentrated H_2SO_4 and 1 ml sodium azide solution (2 g NaN_3 in 100 ml distilled water) to the sample in the DO bottle. Store sample as in method 1. Complete the procedure using 2 ml of manganous sulfate solution, 3 ml alkali iodide solution, and 2 ml of concentrated H_2SO_4 .

3.2 If either preservation technique 1 or 2 is employed, complete the analysis within 4-8 hours after sampling.

4. Interferences

4.1 The azide modification is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution, or which are oxidized by free iodine in an acid solution; (d) domestic sewage; (e) biological flocs; and (f) where sample color interferes with end-point detection.

In instances where the azide modification is not applicable, a DO probe will be used.

5. Apparatus

5.1 Sample Bottles - 300 ml $\pm$ 3 ml capacity BOD incubation bottles with tapered ground glasspointed stoppers and flared mouths.

5.2 Pipets - with elongated tips capable of delivering 2.0 ml $\pm$ 0.1 ml of reagent.

6. Reagents

6.1 Manganese sulfate reagent is prepared as follows: Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter and dilute to 1 liter. The manganese sulfate solution should be free of oxidizing impurities which liberate iodine from a KI solution.

6.2 Alkali iodide-azide reagent is prepared as follows: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water

and dilute to 1 liter. Add to the above solution 10 g NaN_3 dissolved in 40 ml of distilled water. The solution should be free of oxidizing impurities on acidification with sulfuric acid.

6.3 Sulfuric acid, concentrated.

6.4 Starch solution is prepared as follows: Prepare an emulsion of 10 g soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10°C refrigerator. Dry, powdered starch indicators such as "thyodene" may be used in place of starch solution.

6.5 Potassium fluoride solution is prepared as follows: Dissolve 40 g $\text{KF} \cdot 2\text{H}_2\text{O}$ in distilled water and dilute to 100 ml.*

6.6 Sodium thiosulfate stock solution, (0.75N), is prepared as follows: Dissolve 186.15 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

6.7 Standard sodium thiosulfate titrant, (0.0375N), is prepared as follows: Prepare by diluting 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standard sodium thiosulfate, exactly 0.0375N, is equivalent to 0.300 mg of DO per 100 ml. Standardize with 0.0375N potassium dichromate.

* Used to eliminate ferric iron above levels of 10 mg/liter.

6.8 Standard potassium acid iodate, (potassium bi-iodate, potassium biniodate), (0.0375N), is prepared as follows: Dissolve 1.2184 $\text{KH}(\text{IO}_3)_2$ primary standard grade in 1 liter of distilled water. The solution must be prepared in a volumetric flask.

6.9 Standardization of 0.375N sodium thiosulfate is prepared as follows: Dissolve $2 \text{ g} \pm 0.1 \text{ g}$ KI in 100-150 ml distilled water; add 10 ml of 10% H_2SO_4 followed by 20 ml standard $\text{KH}(\text{IO}_3)_2$ (see Note a). Dilute to 300 ml and titrate with the standard sodium thiosulfate titrant to a pale straw color. Add 1-2 ml starch solution and continue the titration drop by drop until the blue color disappears. Run in duplicate. Duplicate determination should agree within 0.05 ml.

7. Procedure

7.1 To the sample collected in the BOD incubation bottle add 2 ml of the manganous sulfate solution followed by 2 ml of the alkali-iodide-azide reagent, well below the surface of the liquid; stopper with care to exclude air bubbles and mix well by inverting the bottle several times. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again. With estuarine-type waters, a minimum 2-minute period of contact with the precipitate rather than settling is sufficient. When settling has produced at least 100 ml of clear supernate, carefully remove the stopper and immediately add 2.0 ml of concentrated H_2SO_4 (sulfamic acid packets, 3 g, may be substituted for H_2SO_4) by allowing the acid to run down the neck of the bottle,

re-stopper and mix by gentle inversion until the iodine is uniformly distributed through the bottle. Complete the analysis within 45 minutes. (See Note b)

7.2 Transfer the entire bottle contents by inversion into a 500 ml wide-mouth or Erlenmeyer flask and titrate with 0.0375N thiosulfate solution (where problems of stability arise, 0.0375N PAO may be substituted as titrant) to a pale straw color. Add 1-2 ml of starch solution or 0.1 g of powdered indicator and continue to titrate to the first disappearance of the blue color.

7.3 If ferric iron is present (100-200 ppm), add 1.0 ml of KF solution before acidification.

7.4 Occasionally a dark brown or black precipitate persists in the bottle after acidification. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few more drops of H_2SO_4 will effect dissolution.

8. Calculation

8.1 Each ml of 0.0375N sodium thiosulfate titrant is equivalent to 1 mg/l DO when the entire bottle contents are titrated.

8.2 If the results are desired in milliliters of oxygen gas per liter at 0°C and 760 mm pressure, multiply mg/l DO by 0.698.

8.3 To express the results as percent saturation at 760 mm atmospheric pressure, the solubility data in Table 25 (Whipple & Whipple Table, Page 409. Standard Methods, 12th Edition) may be used. Equations

for correcting the solubilities to barometric pressures other than mean sea level are given below the Table.

8.4 The solubility of DO in distilled water at any barometric pressure, P (mm Hg), temperature, T°C, and saturated vapor pressure, U (mm Hg), for the given T, may be calculated between the temperature of 0 and 30°C by:

$$\text{ml/l DO} = \frac{(P - U) \times 0.678}{35 + T}$$

and between 30 and 50°C by:

$$\text{ml/l DO} = \frac{(P - U) \times 0.827}{49 + T}$$

Note (a) The volume of standard bi-iodate to be employed in the standardization procedure may be reduced to 9 ml to accommodate the use of a 10 ml burette.

Note (b) The mixing should produce a fine flocculent precipitate when manganous sulfate and alkali-iodide-azide is added. If the precipitate forms in large globules, shake well to disperse globules.

REFERENCES

Kroner, R. C., Longbottom, J. E., and Gorman, R. A Comparison of Various Reagents Proposed for Use in Winkler Procedure for Dissolved Oxygen, PHS Water Pollution Surveillance System, Applications and Development, Report No. 12. Water Quality Section, Basic Data Branch, July, 1964.

Mancy, K. H., and Jaffe, T. Analysis of Dissolved Oxygen in Natural and Waste Waters, U.S. D. H. E. W., PHS Pub. No. 999WP-37, April, 1966.

NITROGEN-NITRATE

Manual Method

1. Scope

1.1 This method is applicable to a wide variety of samples. The principle of the method is based upon the reaction of the nitrate ion with brucine sulfate in a 13N H_2SO_4 solution at about 100°C. The optical density of the resulting colored complex is measured at 410 m μ .

1.2 Applicable range is 0.1 to 2 mg $\text{N-NO}_3/\text{l}$.

2. Sampling

2.1 Samples should be collected in glass or plastic containers and analyzed immediately.

3. Preservation

3.1 Until more conclusive data is obtained, samples may be preserved as follows:

3.1.1 Addition of 40 mg Hg/l (as HgCl_2) and stored at 4°C.

3.1.2 Addition of 1 m ^{3} of CHCl_3 /100 ml sample. Store at 4°C.

3.1.3 Freezing.

Note (a) -- Data available at the present time indicate that in using the above methods the nitrate concentration is stable over a period of at least six weeks.

4. Interferences

4.1 Dissolved organic matter will cause an off color in 13N H_2SO_4 and must be compensated for by additions of all reagents except the brucine-sulfanilic acid reagent. This also applies to natural color present not due to dissolved organics.

4.2 If turbidity is present, it must be removed by prior filtration, preferably through a 0.45 μ membrane filter.

4.3 The effect of salinity is eliminated by addition of sodium chloride to the blanks, standards and samples.

4.4 All strong oxidizing or reducing agents interfere. The presence of oxidizing agents may be determined by the addition of orthotolidine reagent.

4.5 Residual chlorine interference is eliminated by the addition of sodium arsenite.

4.6 Ferrous and ferric iron and quadrivalent manganese give slight positive interference, but in concentrations less than 1 mg/l these are negligible.

5. Apparatus

5.1 Spectrophotometer or filter photometer suitable for measuring optical densities at 410 m μ and capable of accommodating 25 mm diameter cells.

5.2 Sufficient number of 25 mm diameter matched tubes for reagent blanks, standards, and samples.

5.3 Neoprene coated wire racks to hold 25 mm diameter tubes.

5.4 Water bath suitable for use at 100°C. This bath should contain a stirring mechanism so that all tubes are at the same temperature.

5.5 Water bath suitable for use at 10-15°C.

6. Reagents

6.1 Sodium chloride solution, (300 g/l), is prepared as follows:
Dissolve 300 g NaCl in distilled water and dilute to 1000 ml.

6.2 Sulfuric acid solution is prepared as follows: Carefully add 500 ml H_2SO_4 (sp gr 1.84) to 125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.

6.3 Brucine-sulfanilic acid reagent is prepared as follows: Dissolve 1 g brucine sulfate ($\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4$) $\cdot \text{H}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ and 0.1 g sulfanilic acid ($\text{NH}_2\text{C}_6\text{H}_4\text{SO}_3\text{H} \cdot \text{H}_2\text{O}$) in 70 ml hot distilled water. Add 3 ml concentrated HCl, cool, mix and dilute to 100 ml. Store in a dark bottle at 5°C. This solution is stable for several months; the pink color that develops slowly does not effect its usefulness. Mark bottle with a warning:

CAUTION: Brucine sulfate is toxic; take care to avoid ingestion.

6.4 Potassium nitrate stock solution, (1 ml = 0.1 mg $\text{NO}_3\text{-N}$), is prepared as follows: Dissolve 0.7218 g anhydrous KNO_3 in distilled water and dilute to 1 liter.

6.5 Potassium nitrate standard solution, (1 ml = 0.01 mg $\text{NO}_3\text{-N}$), is prepared as follows: Dilute 100 ml of the stock solution to 1 liter. This standard solution should be prepared fresh weekly.

6.6 Acetic acid, (1 + 3), is prepared as follows: Dilute 1 vol. glacial CH_3COOH with 3 vol. distilled water.

7. Procedure

7.1 Adjust the pH of the samples to approximately pH 7 with 1 + 3 acetic acid and if necessary filter through a 0.45 μ membrane filter or Whatman No. 42 filter paper depending on the amount and

type of suspended solids present.

7.2 Set up the required number of matched tubes in the rack to handle reagent blank, standards, and samples. If it is necessary to correct for color or dissolved organic matter (which will cause color on heating), a set of duplicate tubes must be used to which all reagents except the brucine-sulfanilic acid have been added.

7.3 Pipet 10 ml or an aliquot of the samples diluted to 10 ml into the sample tubes.

7.4 If the samples are saline, add 2.0 ml of the 30% NaCl solution to the reagent blank, standards, and samples. For fresh water samples NaCl may be omitted. Mix contents of tubes by swirling and place rack in cold water bath (0-10°C).

7.5 Pipet 10 ml of H_2SO_4 solution into each tube and mix by swirling. Allow tubes to come to thermal equilibrium in the cold bath.

7.6 Add 0.5 ml brucine-sulfanilic acid reagent to each tube (except the interference control tubes) and carefully mix by swirling; then, place the rack of tubes in the boiling water bath for exactly 25 minutes.

7.7 Remove rack of tubes from the hot water bath and immerse in the cold water bath and allow to reach thermal equilibrium (20-25°C).

7.8 Dry tubes and read optical density against the reagent blank at 410 $m\mu$.

8. Calculation

8.1 Obtain a standard curve by plotting the optical densities of standards run by the above procedure against mg $\text{NO}_3\text{-N}$. The color reaction does not always follow Beer's law.

8.2 Subtract the absorbance of the sample without the brucine-sulfanilic reagent from the absorbance of the sample containing brucine-sulfanilic acid and read the resultant absorbance in mg $\text{NO}_3\text{-N}$. Multiply by factor for converting mg per aliquot of sample to mg per liter.

$$\text{mg/l NO}_3\text{-N} = \frac{1000}{\text{ml sample}} \times \text{mg NO}_3\text{-N from curve}$$

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Jenkins, D., and Medsker, L. Brucine method for the determination of nitrate in ocean, estuarine, and fresh waters. Analytical Chemistry, 36(3):610-612. 1964.

Kahn, L., and Brezenski, F. T. Determination of nitrate in estuarine waters. Environmental Science and Technology, 1:488-492. 1967.

Mullin, J. B., and Riley, J. P. The spectrophotometric determination of nitrate in natural waters in particular reference to sea water. Analytica Chimica Acta, 12:464-480. 1955.

Manual Method

1. Scope

1.1 This method is applicable for the determination of nitrite ion in water and wastewater.

1.2 The diazonium compound formed by diazotation of sulfanilamide by nitrite ion in water under acid conditions is coupled with N (1-naphthyl) ethylenediamine to produce a reddish-purple color which is read in a spectrophotometer at 520 m μ .

2. Sampling

2.1 Samples shall be collected in glass or plastic bottles of suitable size. All bottles must be thoroughly cleansed and thoroughly rinsed to remove soluble materials from containers.

3. Preservation

3.1 Samples should be preserved at the time of collection with 1 ml of chloroform per 100 ml of samples or 40 mg Hg/ml as mercuric chloride. Store at 4°C. DO NOT PRESERVE WITH SULFURIC ACID.

4. Interferences

4.1 There are very few known interferences at concentrations less than 1000 times that of the nitrite; however, recent addition of strong oxidant or reductants to the sample will readily affect the nitrite concentration.

5. Apparatus

5.1 Spectrophotometer or filter photometer suited for use in the range of 515 to 530 m μ .

6. Reagents

6.1 Color reagent is prepared as follows: To 400 ml of distilled water, while stirring on a magnetic mixer, add 50 ml conc. HCl, 5.0 g of sulfanilamide and 0.5 g of N (1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved and dilute to 500 ml.

6.2 Sodium acetate buffer, (2M), is prepared as follows: To 50 ml, while stirring on a magnetic mixer, add 27.4 g of $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$. Stir until dissolved and dilute to 100 ml.

6.3 Nitrite nitrogen stock standard, (100 mg/l NO_2^-), is prepared as follows: Dissolve 0.4926 g sodium nitrite, anhydrous NaNO_2 , and dilute to 1000 ml with distilled water. Preserved with 2 ml chloroform per liter, this solution will remain stable for one week.

7. Procedure

7.1 If the sample has a pH greater than 10, adjust it to approximately pH 7 with acetic acid (1:3).

7.2 Filter the sample through a Whatman No. 42 paper or .45 μ membrane filter, depending on the amount of suspended solids in the sample.

7.3 To 40 ml (or an aliquot diluted to 40 ml) add 1.0 ml color reagent and 1.0 ml of buffer. Dilute to 50 ml mark and mix.

7.4 Read the color after 10 to 30 minutes in the spectrophotometer at 520 m μ against reagent blank in the same size cells as used with nitrite nitrogen standards.

7.5 Prepare in 50 ml Nessler tubes a reagent blank and five standards in the desired concentration range from the 100 mg/l NO_2^- -N standard and

dilute to about 40 ml. The slope of the curve should be checked each day with a blank and three standards.

7.6 To each, add 1.0 ml of color reagent and 1.0 ml of acetate buffer. Dilute to 50 ml and allow color to develop for 10 minutes. The color reaction medium should be between pH 2.0 and 2.5.

7.7 An estimate of the color density which will permit approximately 25% transmission in various size cells can be obtained from the following:

ml stock standard	mg/50 ml NO ₂ -N	mg/l NO ₂ -N	cell length cm
2.0	.002	.040	10.
4.0	.004	.080	5.
20.0	.020	.40	1.

7.8 Read in spectrophotometer at 520 mμ against the reagent blank.

8. Calculation

8.1 The colorimetric reaction obeys Beer-Lambert's law so that in plotting the absorbancy of the standards against the concentration, a straight line results.

8.2 Calculate the concentration of nitrite nitrogen ion in the sample in mg/l as follows:

$$\text{mg/l NO}_2\text{-N} = \text{absorbance of sample} \times \frac{\text{mg/l standard}}{\text{absorb. std.}} \times \frac{1000}{\text{ml sample}}$$

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NITRATE-NITRITE

Automated Method A

1. Scope

1.1 This method is applicable to water samples which contain less than 500 mg/l calcium. The applicable range of this method is 0.05-10 mg/l nitrite or nitrate nitrogen. Approximately 20 samples per hour can be analyzed.

1.2 This procedure, using the Technicon Autoanalyzer, determines $\text{NO}_2\text{-N}$ by the conventional diazotization-coupling reaction. The $\text{NO}_3\text{-N}$ is reduced with hydrazine sulfate in another portion of the sample and the nitrite thus formed is determined in the usual manner.

1.3 Subtraction of the $\text{NO}_2\text{-N}$ originally present in the sample from the total $\text{NO}_2\text{-N}$ found will give the original $\text{NO}_3\text{-N}$ concentration in terms of $\text{NO}_2\text{-N}$.

2. Sampling

2.1 Same as in the manual method

3. Preservation

3.1 Same as in the manual method

4. Interferences

4.1 The following table lists the concentration of ions that cause no interference in the determination of nitrite and nitrate nitrogen. The same interfering ion concentration applies to either nitrite or nitrate:

<u>Ion</u>	<u>Mg/l ion not causing interference</u>
Cl ⁻	30,000
PO ₄ ⁻³	50
S ⁻²	Note
NH ₃ -N	80
Mg ⁺²	75
Ca ⁺²	240
Fe ⁺³	30
ABS	60

Note -- The apparent NO₃-N and NO₂-N concentrations varied +10% with concentrations of sulfide ion up to 10 mg/l.

5. Apparatus

5.1 A Technicon Autoanalyzer consisting of the following: two proportional pumps, two colorimeters, each with an 8 mm flow-through cell and 520 mμ filters, one continuous filter, one sampler (large), two recorders, one 38°C temperature bath, and two time delay coils.

6. Reagents

6.1 Color developing reagent is prepared as follows: To approximately 3 liters of distilled water add 400 ml concentrated phosphoric acid (sp g 1.834), 60 g sulfanilamide (H₂N-C₆H₄ SO₂ NH₂) followed by 3.0 g N(1-naphthyl)ethylenediamine dihydrochloride. Dilute the solution to 4 liters with distilled water and store in a dark bottle in the refrigerator. This solution is stable for approximately 1 month.

Note -- It may be necessary to apply heat in order to dissolve the sulfanilamide.

6.2 Copper sulfate, stock solution, is prepared as follows: Dissolve 2.5 g of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in distilled water and dilute to 1 liter.

6.3 Copper sulfate, dilute solution, is prepared as follows: Dilute 20 ml of stock solution to 2 liters with distilled water.

6.4 Sodium hydroxide, stock solution, (10N), is prepared as follows: Dissolve 400 g NaOH in 750 ml distilled water, cool, and dilute to 1 liter.

6.5 Sodium hydroxide, (1.0N), is prepared as follows: Dilute 100 ml of stock NaOH solution to 1 liter.

6.6 Sodium hydroxide, (0.3N), is prepared as follows: Dilute 60 ml of stock NaOH solution to 2 liters.

6.7 Hydrazine sulfate solution is prepared as follows: Dissolve 54.92 g of hydrazine sulfate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$) in 1800 ml of distilled water and dilute to 2000 ml. This solution is stable for approximately 6 months. CAUTION: Toxic if ingested. Mark container with warning.

6.7.1 Working hydrazine solution is prepared as follows: Using distilled water, dilute 90 ml of stock solution (6.7) to four liters. This dilute working solution is stable for several months without special precaution.

6.8 Nitrate standards

6.8.1 Potassium nitrate stock standard, (1000 mg/l $\text{NO}_3\text{-N}$), is prepared as follows: Dissolve 7.2180 g of KNO_3 , oven-dried at 100-105°C for 2 hours, in distilled water and dilute to 1000 ml. Add 1 ml chloroform as a preservative. This solution is stable for 6 months.

6.8.2 Potassium nitrate dilute standard, (100 mg/l $\text{NO}_3\text{-N}$), is prepared as follows: Dilute 50 ml of stock KNO_3 solution to 500 ml in a volumetric flask. From this dilute solution prepare the following standards in 500 ml volumetric flasks:

<u>mg/l $\text{NO}_3\text{-N}$</u>	<u>ml standard solution</u>
0.4	2.0
1.0	5.0
1.6	8.0
3.0	15.0
5.0	25.0
7.0	35.0
10.0	50.0

6.9 Nitrite standards

6.9.1 Potassium nitrite stock standard, (1000 mg/l $\text{NO}_2\text{-N}$), is prepared as follows: Dissolve 4.9260 g NaNO_2 , oven-dried at 100-105°C for two hours, in distilled water and dilute to 1000 ml. Add 1 ml chloroform as a preservative. Store in the refrigerator. This solution is stable for one month.

6.9.2 Potassium nitrite, dilute standard, (100 mg/l), is prepared as follows: Dilute 50 ml of stock NaNO_2 solution to 500 ml in a volumetric flask. From this dilute solution

prepare the same volumetric standards as in 7.3 below.

Prepare this solution fresh each week.

7. Procedure

7.1 Set up the manifold as shown in Figures 1 and 2. Allow both colorimeter (with the proper filters) and recorder to warm up for 30 minutes; then, run a base line with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on each colorimeter. Adjust base line to 0.01 optical density. Place a distilled water wash tube in alternate openings on the sampler and set sample timing at 1.5 minutes.

7.2 Run a 2.0 mg/l $\text{NO}_3\text{-N}$ and a 2.0 mg/l $\text{NO}_2\text{-N}$ standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height. If the $\text{NO}_3\text{-N}$ peak is lower than that of the $\text{NO}_2\text{-N}$ peak, the temperature of the reduction bath should be increased until they are equal. If the $\text{NO}_3\text{-N}$ peak is higher than the nitrate, the temperature should be reduced. When the correct temperature of the bath has been determined, no further adjustment should be necessary.

7.3 Arrange standards in sampler in $\text{NO}_2\text{-N}$ - $\text{NO}_3\text{-N}$ order with increasing concentration of nitrogen. Place unknown samples in sampler tubes and place in alternate openings of sampler. An $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ standard of equal nitrogen concentration should be placed after every 10 samples as a further check on the system and to more easily identify peaks.

8. Calculation

8.1 Description of computational procedures is as follows:

A = Absorbance from peak and base line heights:

8.1.1 In all cases, compute absorbance (or a number directly proportional to it) as,

$$A = -\log \left(1 - \frac{p}{w-b} \right) \quad \text{where:}$$

p = peak height in cm (a function of concentration)

b = base line height in cm

w = chart width in cm (28)

8.2 Slope of regression line, $A = f(C)$:

$$S = \frac{\sum_{i=1}^n A_i C_i}{\sum_{i=1}^n C_i^2}$$

where: n = number of standards such that

$$C_{\text{sample}} = \frac{A_{\text{sample}}}{S}$$

C = concentration of standards

8.3 Calculation of sample concentration:

$$C_d = \frac{A}{S}$$

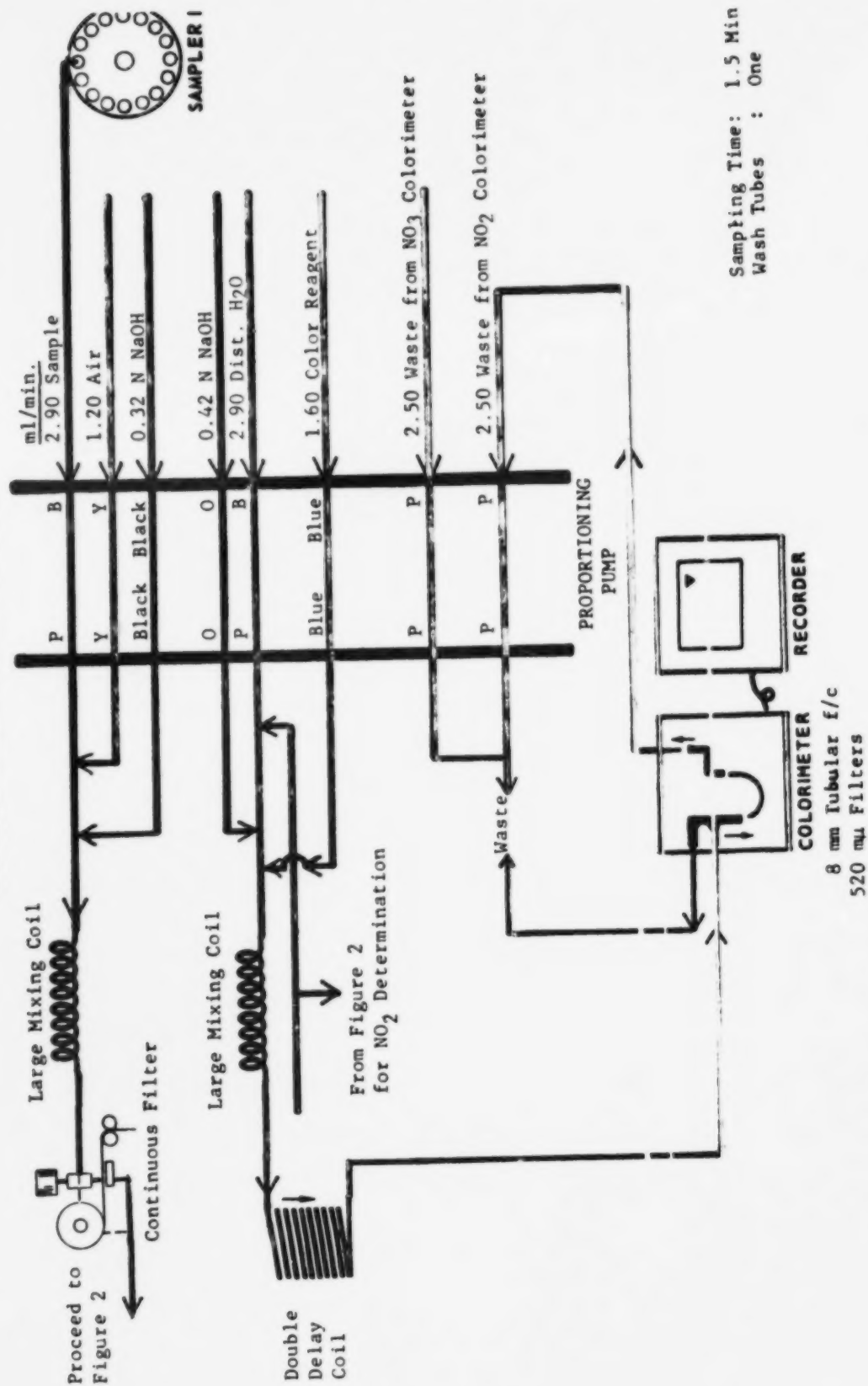
$$C_o = C_d f$$

where: C_d = concentration of diluted sample

C_o = concentration of original sample

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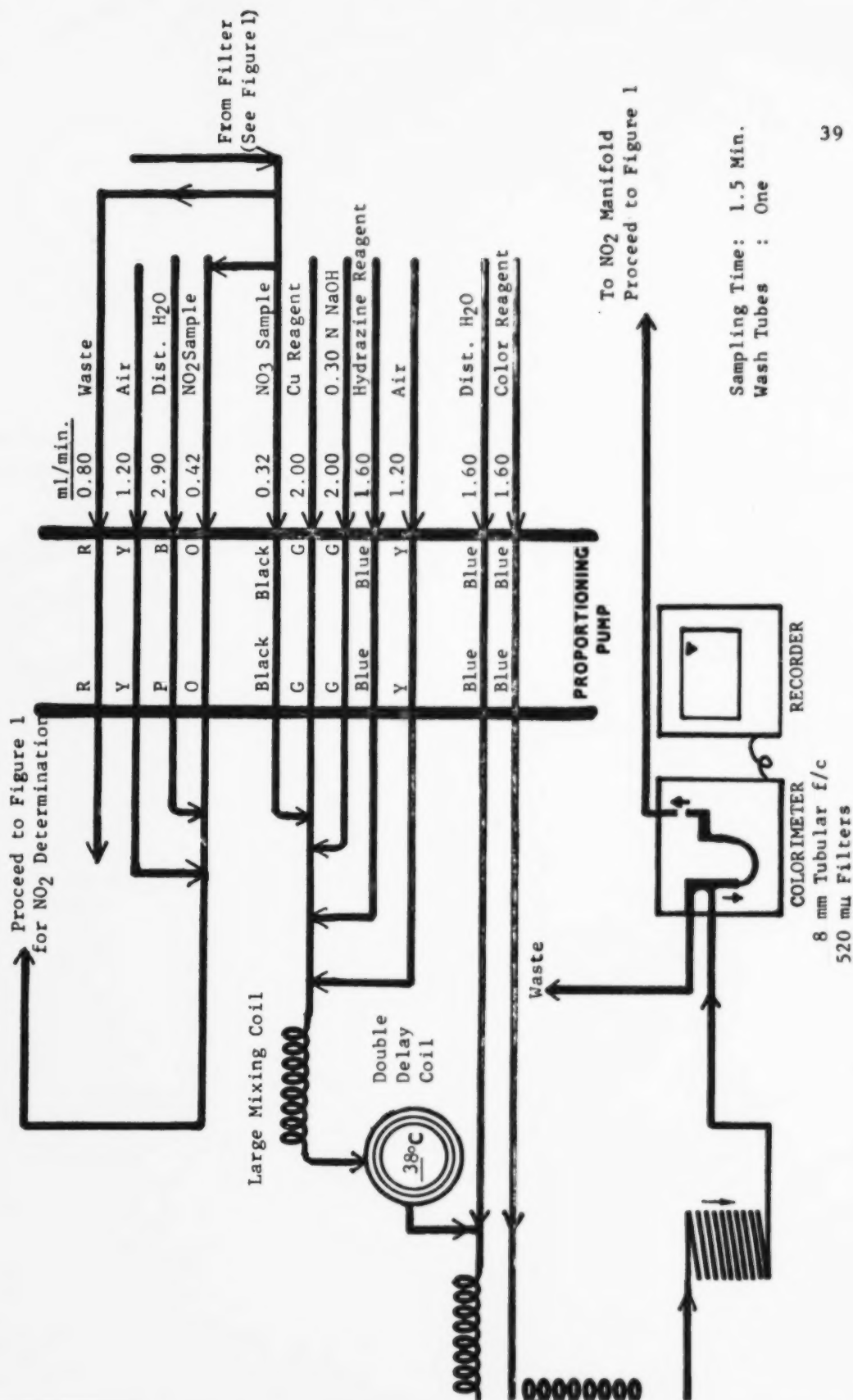
Figure 1. Nitrate-Nitrite Manifold



Sampling Time: 1.5 Min
Wash Tubes : One

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Figure 2. Nitrate-Nitrite Manifold



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NITROGEN-AMMONIA
Distillation Method

1. Scope

1.1 This distillation method covers the determination of ammonia nitrogen exclusive of total Kjeldahl nitrogen in surface waters.

1.2 All reagents and standards are to be prepared with ammonia-free water. Such water is best prepared by the passage of distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. These resins should also be selected so that organic compounds which might subsequently interfere with the ammonia determination will be removed.

1.3 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is then distilled into a solution of boric acid. The ammonia in the distillate can be determined either colorimetrically by Nesslerization or titrimetrically with standard sulfuric acid with the use of a mixed indicator, the choice between these two procedures depending on the concentration of the ammonia.

2. Sampling

2.1 Samples shall be collected in glass or plastic bottles of suitable size. All bottles must be thoroughly cleansed and thoroughly rinsed to remove soluble material from containers.

3. Preservation

3.1 Until more conclusive data are obtained, samples may be preserved by the addition of 40 mg Hg^{++} per liter.

4. Interferences

4.1 A number of aromatic and aliphatic amines, as well as other compounds, both organic and inorganic, will cause turbidity upon the addition of Nessler reagent; consequently, direct Nesslerization has been discarded as a method. Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out. Volatile alkaline compounds such as hydrazine will influence the titrimetric results. Some volatile compounds, such as certain ketones, aldehydes, and alcohols, may cause an off-color upon Nesslerization in the distillation method. Some of these, such as formaldehyde, may be eliminated by boiling off at a low pH prior to distillation and Nesslerization. Residual chlorine must also be removed by pretreatment of the sample with sodium thiosulfate before distillation.

5. Apparatus

5.1 An all-glass distilling apparatus with an 800-1000 ml flask.

5.2 Spectrophotometer or filter photometer for use at 425 m μ and providing a light path of 1 cm or more.

5.3 Nessler tubes: Matched Nessler tubes (APHA Standard) about 300 mm long, 17 mm inside diameter, and marked at 225 mm $\pm$ 1.5 mm inside measurement from bottom.

5.4 Erlenmeyer flasks: The distillate is collected in 500 ml glass-stoppered flasks. These flasks should be marked at the 350 and the 500 ml volumes. With such markings, it is not necessary to transfer

the distillate to volumetric flasks. For analysis where levels are low and the precision required is high, collect the distillate in volumetric flasks and bring to volume.

6. Reagents

6.1 Ammonium chloride, standard solution, ($1\text{ ml} = 0.01\text{ mg NH}_3\text{-N}$), is prepared as follows: Dissolve 3.819 g NH_4Cl in water and bring to volume in a 1 liter volumetric flask for use as a stock solution. Dilute 10 ml of this stock solution to 1 liter in a volumetric flask for use as the standard ammonium chloride solution.

6.2 Boric acid solution, (20 g/l), is prepared as follows: Dissolve 20 g H_3BO_3 in water and dilute to 1 liter.

6.3 Mixed indicator is prepared as follows: Mix 2 volumes of 0.2% methyl red in 95% ethyl alcohol with 1 volume of 0.2% methylene blue in 95% ethyl alcohol. This solution should be prepared fresh every 30 days (see Note a).

6.4 Nessler reagent is prepared as follows: Dissolve 100 g of mercuric iodide and 70 g of potassium iodide in a small amount of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 ml of water. Dilute the mixture to 1 liter. If this reagent is stored in a Pyrex bottle out of direct sunlight, it will remain stable for a period of up to 1 year (see Note b).

6.5 Borate buffer is prepared as follows: Add 88 ml of 0.1N NaOH solution to 500 ml of 0.025M sodium tetraborate solution (5.0 g $\text{Na}_2\text{B}_4\text{O}_7$ per liter) and dilute to 1 liter.

6.6 Sulfuric acid, standard solution, (0.02N, 1 ml = 0.28 mg $\text{NH}_3\text{-N}$), is made by preparing a stock solution of approximately 0.1N acid by diluting 3 ml of concentrated H_2SO_4 (sp gr 1.84) to 1 liter with CO_2 -free distilled water. Dilute 200 ml of this solution to 1 liter with CO_2 -free distilled water. Standardize the approximately 0.02N acid so prepared against 0.0200N Na_2CO_3 solution. This last solution is prepared by dissolving 1.060 g anhydrous Na_2CO_3 , oven-dried at 140°C , and diluting to 1 liter with CO_2 -free distilled water (see Note c).

6.7 Sodium hydroxide, (1N), is prepared as follows: Dissolve 40 g NaOH in ammonia-free water and dilute to 1 liter.

6.8 Dechlorinating reagents - a number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

6.8.1 Sodium thiosulfate (1/70N): Dissolve 3.5 g $\text{Na}_2\text{S}_2\text{O}_3$ in ammonia-free water and dilute to 1 liter. One ml of this solution will remove 1 mg/l of residual chlorine in 500 ml of sample.

6.8.2 Sodium arsenite (1/70N): Dissolve 1.0 g NaAsO_2 in ammonia-free water and dilute to 1 liter.

7. Procedure

7.1 Preparation of equipment should take place as follows: Add 500 ml of ammonia-free water to an 800 ml Kjeldahl flask. The addition of boiling chips which have been previously treated with dilute NaOH will

prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

7.2 Sample preparation is as follows: To 400 ml of sample add 1.0N NaOH until the pH is 9.5, checking the pH during addition with a pH meter.

7.3 Distillation should be done using the following procedure: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 ml Kjeldahl flask and add 25 ml of the borate buffer. Distill 300 ml at the rate of 6-10 ml/min. into 50 ml of 2% boric acid contained in a 500 ml glass-stoppered Erlenmeyer flask. Dilute the distillate to 500 ml in the flask and Nesslerize an aliquot to obtain an approximate value of the ammonia nitrogen concentration. For concentrations above 1.0 mg/l the ammonia should be determined titrimetrically. For concentrations below this value it is determined colorimetrically.

7.4 Determination of ammonia in distillate can be found as follows: Determine the ammonia content of the distillate either titrimetrically or colorimetrically as described below (see 7.4.1 and 7.4.2).

7.4.1 Titrimetric determination is as follows: Add 3 drops of the mixed indicator to the distillate and titrate the ammonia with the 0.02N H_2SO_4 , matching the end point against a blank containing the same volume of ammonia-free water and H_3BO_3 solution.

7.4.2 Colorimetric determination is as follows: Prepare a series of 14 Nessler tubes containing the following volumes of the

standard solution: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, and 4.0 ml. Dilute each tube to 50 ml with ammonia-free water. Add 1 ml of Nessler reagent and mix. After 20 minutes read the optical densities at 425 m μ against a compensating blank of Nesslerized ammonia-free water. From the values obtained prepare a standard curve. Determine the ammonia in the distillate by Nesslerizing 50 ml or an aliquot diluted to 50 ml and reading the optical density at 425 m μ as described above for the standards.

Ammonia nitrogen content is read from the standard curve.

8. Calculation

8.1 Titrimetric

$$\text{mg/l NH}_3\text{-N} = \frac{(\text{ml}_{\text{sample}}^* - \text{ml}_{\text{blank}}) \times 0.28 \times 1000}{\text{sample size}}$$

* Normality of acid is 0.02

8.2 Spectrophotometric

$$\text{mg/l NH}_3\text{-N} = (A_c - A_b) \times \frac{c}{A_s} \times \frac{\text{ml}_d \times D_f \times 1000}{\text{ml}_c \times \text{ml}_k} \quad \text{in which:}$$

A_c = Absorbance sample

ml_d = ml distilled

A_b = Absorbance blank

ml_c = ml sample

A_s = Absorbance standards

ml_k = ml for color development

c = Concentration of standards

D_f = Dilution factor

Note (a) Specially denatured ethyl alcohol conforming to Formula 3A or 30 of the U. S. Bureau of Internal Revenue may be substituted for 95% ethanol.

Note (b) This reagent should give the characteristic color with ammonia within 10 minutes after addition, and should not produce a precipitate with small amounts of ammonia (0.04 mg in a 50 ml volume).

Note (c) An alternate and perhaps preferable method is to standardize the approximately 0.1N H_2SO_4 solution against a 0.100N Na_2CO_3 solution. By proper dilution the 0.0200N acid can then be prepared.

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NITROGEN-AMMONIA

Automated Method

1. Scope

1.1 Ammonia reacts with alkaline phenol and sodium hypochlorite to produce a blue color thought by some to be an indole phenol. An advantage of the reaction is the formulation of a truly soluble color product. The sensitivity approximates that of the Nessler reaction. Iron, chromium, and manganous ions catalyze the reaction; whereas copper ions and the presence of oxidants tend to inhibit color development. Temperature is an important factor in color reproducibility.

1.2 The manifold is set up according to the flow diagram. The sample stream segmented by air is joined with the EDTA- sodium citrate and alkaline phenol stream and passed through a double mixing coil. The stream is then passed through a heating bath controlled by a power-stat operating from a regulated voltage supply. The temperature of the bath should be precisely maintained at a temperature near 85°C. The color reaction is halted by passing the heated stream through a jacketed mixer cooled with tap water. The stream then passes on to the colorimeter where the optical density is measured at 630 mμ in a 50 mm cell and recorded on a recorder at IX range expansion for a permanent record.

2. Sampling

2.1 Sample collection is the same as in the ammonia nitrogen manual method.

3. Preservation

3.1 Sample preservation is the same as in the ammonia nitrogen manual method.

4. Interferences

4.1 Most interferences are overcome by the use of the EDTA - sodium citrate as a chelating agent.

5. Apparatus

5.1 Technicon Autoanalyzer consisting of the following: a sampler II, proportioning pump, two double mixing coils, one adjustable heating bath with a full-time delay coil, a variable auto-transformer (powerstat), a jacketed mixing coil with nipples for cooling water, a colorimeter with 50 mm flow cells, 630 m μ interference filters, red-sensitive photocells, recorder, and plastic sample cups.

6. Reagents

6.1 Ammonia-free water is prepared as follows: Pass good quality distilled water through a strong cation exchanger. The Permutit Company markets Folin's Ammonia Permutit for this purpose

6.2 Ammonia standard stock solution, (1 ml = 1 mg NH₃ as N), is prepared as follows: Dissolve 3.319 g NH₄Cl (previously dried at 110°C for two hours) in ammonia-free water and dilute to one liter.

6.3 EDTA - sodium citrate solution is prepared as follows: Add 10 g Na₂EDTA and 5 g Na₃citrate to 800 ml distilled ammonia-free water

and mix until dissolved. Adjust the pH to 11.0 with NaOH. Add 20 ml acetone and dilute quickly to 1000 ml. Store immediately in a tightly-stoppered container to prevent a loss of acetone.

6.4 Alkaline phenol solution is prepared as follows: To 50 ml of water in a 1500 ml beaker add 83 g of phenol crystals. With stirring, add 180 ml of a 20% solution of sodium hydroxide. Dilute to 1000 ml and filter the solution. Store in an amber bottle.

6.5 Sodium hypochlorite solution is prepared as follows: Dilute 88 ml of a 6% solution of sodium hypochlorite (Purex Super Bleach) to one liter.

7. Procedure

7.1 See schematic diagram

8. Calculation

8.1 Description of computational procedures is as follows:

A = Absorbance from peak and base line heights:

8.1.1 In all cases, compute absorbance (or a number directly proportional to it) as,

$$A = -\log\left(1 - \frac{p}{w-b}\right) \quad \text{where:}$$

p = peak height in cm (a function of concentration)

b = base line height in cm

w = chart width in cm (28)

8.2 Slope of regression line, $A = f(C)$:

$$S = \frac{\sum_{i=1}^n A_i C_i}{\frac{\sum_{i=1}^n C_i^2}{n}}$$

where: n = number of standards such that

$$C_{\text{sample}} = \frac{A_{\text{sample}}}{S}$$

C = concentration of standards

8.3 Calculation of sample concentration:

$$C_d = \frac{A}{S}$$

$$C_o = C_d f$$

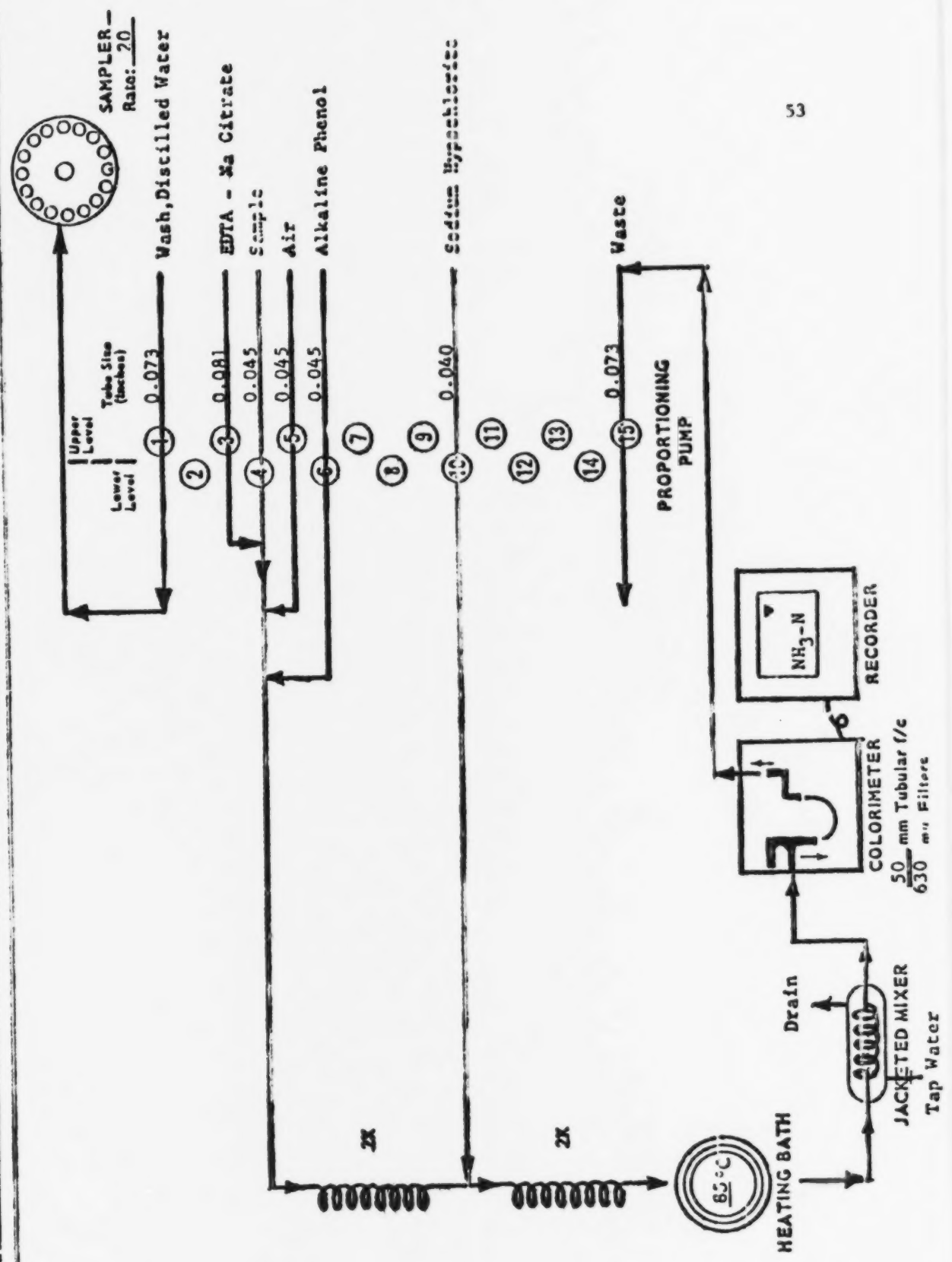
where:

C_d = concentration of diluted sample

C_o = concentration of original sample

ON PAGE... 0.005 to 2.50 mg/l
 LE... Filtered Fresh Water or Sea Water Samples
 NCE...

SIGNATURE
 AFFILIATION



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1. Scope

1.1 This method covers the determination of total Kjeldahl nitrogen in fresh and estuarine waters. The Kjeldahl procedure converts the nitrogen of most components of biological origin, such as amino acids, peptides and proteins, but may not convert the nitrogenous matter in many industrial wastes, such as azines, hydrazones, nitro compounds, oximes, semicarbazones, and some refractory tertiary amines. The titrimetric method is applicable to concentrations above 2 mg N per liter and possibly to lower concentrations if reagent blanks are consistent enough. The Nesslerization method is applicable to concentrations above 0.1 mg N per liter. The lower limit can be extended by concentration of the distillate prior to Nesslerization.

1.1.1 Total Kjeldahl nitrogen is defined as the sum of free ammonia and of organic nitrogen compounds which are converted to $(\text{NH}_4)_2\text{SO}_4$ under the conditions of digestion which are specified below.

1.1.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free ammonia value from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.

1.1.3 Organic nitrogen is defined as the nitrogen derived from all organic substances in the sample and shall include Kjeldahl nitrogen.

2. Sampling

2.1 Samples should be collected in glass or plastic containers that have been cleaned and rinsed. The containers should be checked before use to determine whether material is released from the container which will adversely affect the sample and subsequent analysis.

3. Preservation

3.1 The samples can be preserved for analysis at ice temperature for a period not exceeding 48 hours. Samples can be preserved with 1 ml conc. H_2SO_4 per liter of sample and retained for longer periods provided that the relationship between organic nitrogen and ammonia is not of interest. The H_2SO_4 will retain ammonia in solution; however, it accelerates the conversion of organic nitrogen to ammonia. Samples treated with H_2SO_4 are also subject to gain ammonia from the air if not tightly sealed.

3.2 Samples can also be preserved with mercuric chloride by adding ~~10 ml of 40 mg Hg^{++} /ml~~ ^{40 mg/ml} as mercuric chloride to a liter of sample.

4. Apparatus

4.1 Spectrophotometer - An instrument capable of measuring absorbance at 425 mμ. It is advisable to have available an instrument which can measure absorbance of solution in 1 to 10 cm length cells. In many cases of very small concentrations of nitrogen (organic), a longer light path of absorbance will give more evenly reproducible readings.

4.2 Digestion and Distillation Apparatus - There are various semi-micro apparatus on the market, Aminco, Fisher, etc., equipped with 100 ml

digestion flasks. This type of digestion apparatus ordinarily restricts one to a 50 ml sample as a maximum.

4.3 The regular Kjeldahl digestion and distillation units are macro apparatus; here too, there are several on the market. Here, one is able to use 500 or 800 ml digestion flasks and is limited to a maximum of 400 to 500 ml liquid sample. For solids, sediments, or plant extracts, the macro apparatus should be the one of choice. In many cases, a 2 g sample size will be a limiting factor because of the foaming, etc., that may take place in digestion. (See Note a)

5. Reagents

5.1 Ammonium chloride stock standard solution, (1 ml contains 1 mg $\text{NH}_3\text{-N}$), is prepared as follows: Dissolve 3.819 g of anhydrous NH_4Cl (dried at 100°C) with ammonia-free water in a 1 liter volumetric flask.

5.1.1 Ammonium chloride standard solution, (1 ml contains 0.010 mg of $\text{NH}_3\text{-N}$), is prepared as follows: Transfer 10.0 ml of the stock solution to a 1 liter volumetric flask and dilute to mark with ammonia-free water.
Mix well.

5.1.2 Dilute ammonium chloride standard solution, (1 ml contains 0.005 mg of $\text{NH}_3\text{-N}$), is prepared as follows: Transfer 50.0 ml of the standard ammonium chloride solution (1 ml = 0.010 mg of $\text{NH}_3\text{-N}$) to a 100 ml volumetric flask and dilute to the mark with ammonia-free water.

Mix well. Dilute standard ammonium chloride solution of various concentrations can be made by proper dilution techniques. The same precautions as to ammonia-free water apply.

5.2 Kjeldahl digestion mixture is prepared as follows to make up 2 liters of solution: Dissolve 267.0 g K_2SO_4 in 1300 ml H_2O and 400 ml concentrated H_2SO_4 . Add, with stirring, a solution of 4 g red HgO in 50 ml of 6N H_2SO_4 . Dilute to 2 liters. Use ammonia-free water throughout.

5.3 NaOH - $Na_2S_2O_3$ is prepared as follows to make up 2 liters of solution: Use 1000 g of NaOH (low content of N) plus 32.0 g $Na_2S_2O_3$ (anhydrous) or 50 g of $Na_2S_2O_3 \cdot 5H_2O$. Make up to 2 liters with ammonia-free water.

5.4 Nessler reagent is prepared as follows:

5.4.1 Dissolve 100 g HgI_2 and 70 g KI in 75-100 ml of ammonia-free water.

5.4.2 Dissolve 160 g NaOH in 500 ml of ammonia-free water and cool the solution.

5.4.3 Add 5.4.1 with stirring to 5.4.2 and dilute to 1 liter with ammonia-free water. Allow precipitate to settle.

5.5 Phenolphthalein indicator solution is prepared as follows: Dissolve 0.5 g in 100 ml of 50% ethanol.

5.6 Sulfuric acid, (0.025N), is prepared as follows: Add 0.7 ml of concentrated H_2SO_4 to 1 liter of ammonia-free water.

5.7 Ammonia-free water is prepared as follows: Any precaution for removal of NH_3 from the distilled water may be used, e.g., all glass distillation apparatus, alkaline KMnO_4 distillation, use of Amberlite MB-1 column, or any other exchange resin which will remove nitrogen.

5.8 Methyl red indicator is prepared as follows: Dissolve 1 g methyl red in 200 ml alcohol.

5.9 Sodium hydroxide standard solution is prepared as follows for 1N: Dissolve 40 g sodium hydroxide in ammonia-free distilled water. Cool, dilute to 1 liter, and store in a plastic bottle.

5.9.1 Sodium hydroxide solution, (0.025N), is prepared as follows: Transfer 25 ml of 5.9 (1N NaOH) to a liter flask, add ammonia-free distilled water to liter mark, and standardize against potassium acid phthalate.

6. Procedure

6.1 Semi-micro

6.1.1 Digestion is carried out using the following procedure: Transfer maximum of 50 ml of sample to a 100 ml digestion flask and add 10 ml of the digestion mixture and several Carborundum boiling chips. Carry out blanks, standard, and sample with standard spikes throughout the entire procedure. In many cases of extended sampling it is prudent to add the Carborundum chips and digestion mixture into the flasks followed by sample additions, etc.

Place the flask on the digester and set the heaters on heat (regulate to prevent excessive spattering or foaming) and digest until the solution evaporates to sulfuric acid fumes. Continue heating after fumes and clearing of the solution for at least 20 minutes more. Remove this flask from the digestion apparatus and cool to room temperature. After cooling to about room temperature, the flask should be washed down with approximately 15 ml of ammonia-free water and the flask put back on the digestion rack and heated and concentrated to SO_3 fumes and clarity of solution, taken off the digester, and allowed to cool to room temperature.

6.1.2 Distillation is carried out using the following procedure:

Add 15 ml of water to the flask, rotate to put all salts in solution; no solid cake adhering on the bottom of the flask! Add 2 drops of phenolphthalein indicator, rotate. Submerge the tip of the delivery tube of the distillation apparatus into 10 ml of 0.025N sulfuric acid in a 100 ml beaker. Dampen the neck of the distillation flask with a few drops of water and attach to the distillation apparatus. (Steam from the generator is being bypassed at these points). Introduce 7 ml of NaOH solution to the well at the top of the distillation apparatus. (If more

digestion solution was used, correspondingly, more NaOH solution must be added to release NH_3). Open the stopcock permitting the solution to flow into the distillation flask and rinse the well down 2 or 3 times with a few ml of water. If the solution in the distillation flask does not have some resemblance of a pink color, add more NaOH solution so that a pink basic color develops. Close the stopcock to the well. Introduce steam into the distillation flask. Distill for 5 minutes after the first drop of distillate flows into the receiving beaker containing 10 ml 0.025N H_2SO_4 . [At this point it is wise to smell for ammonia odor coming off as the distillate. If it is not entrapped in the 0.025N H_2SO_4 , add an additional 10 ml of the acid. If this should occur, these additions must be made to the blank, standard, and sample spike distillates.] Lower the beaker containing the distillate so that the tube is removed from the solution. Continue the distillation for at least one minute, and rinse the outside of the tube into the beaker with a small quantity of water. Transfer the entire distillate quantitatively into a 100 ml volumetric flask and dilute to the mark with ammonia-free water. Mix well. Transfer 50 ml of this dilute distillate to a 50 ml Nessler tube.

Add 1.0 ml of Nessler's reagent in turn to the samples and blanks in the Nessler tubes, mix, and allow to stand 10 to 30 minutes for color development (30 minutes if ammonia content is low -- here it may become necessary to use a longer length cell of sample to read in the spectrophotometer). For higher concentrations of ammonia, an aliquot of the distillate must be used in the Nessler tube and made to the 50 ml mark with ammonia-free water. Measure the absorbance at 425 mμ versus water in the reference cell. Subtract the absorbance of the blank from the absorbance of the samples. Refer to an absorbance-concentration graph or calculate the ratio of concentration in mg per liter. The distilled sample may also be back titrated with .025N NaOH using methyl red indicator (see Note b).

6.2 Macro

- 6.2.1 Digestion is carried out using the following procedure: Transfer the maximum size sample (up to 500 ml if liquid, 2-5 g of sediment) into an 800 ml Kjeldahl digestion flask, add 6 to 8 glass beads, 50 ml of digestion solution, and place the flask on the digestion rack. As the material in the flask concentrates (prevent excess foaming or

spattering with a drop of parafin or silicone), it is well to rotate the flask over the heater area 96 degrees every 15 to 20 minutes. The same precautions as to time for clearing and continued digestion are necessary as in the semi-micro digestion. If the organic (black) material and solution do not clarify, an additional amount of digestion mixture is needed. After digestion has been completed, remove the flask from the rack and cool to approximately room temperature. After the flask has cooled to approximately room temperature, wash down the flask with approximately 50 ml of ammonia-free water and redigest to SO_3 fumes and clarity of solution for 20 minutes before removing from the digestion rack. Allow to cool.

- 6.2.2 Distillation is carried out using the following procedure: Add 200 ml water to the flask and rotate briskly to see that all salts go into solution. No solid, salt material must remain precipitated and adhering to the flask; the caked material must be loosened from the flask! Add 5 drops of phenolphthalein indicator; then, add 45-50 ml of NaOH solution for liberation of NH_3 . Rotate the flask to mix materials (evidence of pink, o.k.). Immediately hook up to the distillation apparatus. Catch the distillate in a 250 ml volumetric flask containing

25 ml of 0.025N H_2SO_4 , being sure that the delivery tube is below the surface of the entrapping acid. Distill until the contents in the distilling flask start bumping, or to a volume close to 225 ml in the receiver, whichever comes first. As soon as approximately 200 ml are in the receiving flask, pull the receiving flask out so that the delivery tube of the distillation is not in solution or obstructed in any way. After the distillation has been halted, remove the distillation flask. Wash down the receiving distillation tube into the 250 ml volumetric flask. Make to the 250 ml mark with ammonia-free water. Mix well. Use either 50 ml of the distillate or an aliquot in the Nessler tube and Nesslerize the solution as described earlier and read in the spectrophotometer at 425 mu. The distilled sample may also be titrated with .025N NaOH using methyl red indicator (see Note b).

7. Calculation

7.1 Spectrophotometric

$$\text{mg/l NH}_3\text{-N} = (A_c - A_b) \times \frac{c}{A_s} \times \frac{\text{ml}_d \times D_f \times 1000}{\text{ml}_c \times \text{ml}_k}$$

in which:

- A_c = Absorbance sample
 A_b = Absorbance blank
 A_s = Absorbance standards
 c = Concentration of standards
 ml_d = ml distilled
 ml_c = ml sample
 ml_k = ml for color development
 D_f = Dilution factor

7.2 Titrimetric

$$\text{mg/l NH}_3\text{-N} = \frac{(\text{ml} \times N) \times 0.28 \times 1000}{\text{sample size}}$$

Note (a) It is necessary that digestion apparatus be provided with good suction take-off to remove water vapor and sulfur trioxide fumes. In some cases it may be necessary to add an additional quantity of digestion reagent ($\text{H}_2\text{SO}_4 \cdot \text{HgSO}_4 \cdot \text{K}_2\text{SO}_4$) to complete digestion. If this occurs, one should further compensate for this by additional NaOH solution in the distillation step for liberation of the NH_3 .

Note (b) Clean the distillation apparatus before use by passing steam through the assembly for about 15 to 30 minutes. The water used for steam generation must be ammonia-free water.

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1. Scope

1.1 These methods cover the determination of specified forms of phosphorus in surface waters, saline waters, industrial waters, and sewage-type water samples. They may be applicable to sediment-type samples, sludges, algal blooms, etc; but sufficient data is not available at this time to warrant such usage when measurements for total phosphorus content are required.

1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, the various forms of phosphorus given in Table 1 may be determined. These forms are, in turn defined in Table 1.

1.2.1 Except for in-depth and detailed studies, the most commonly measured forms of phosphorus are total and soluble phosphorus, and total and soluble orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples; and insoluble forms of phosphorus, as noted, are determined by calculation.

1.3 The methods are usable in the 0.01 to 0.5 mg/l P range.

1.4 Definitions of the various forms of phosphorus are defined in Table 1.

1.4.1 Table 1, phosphorus terminology

A. Unfiltered sample

Total phosphorus - all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

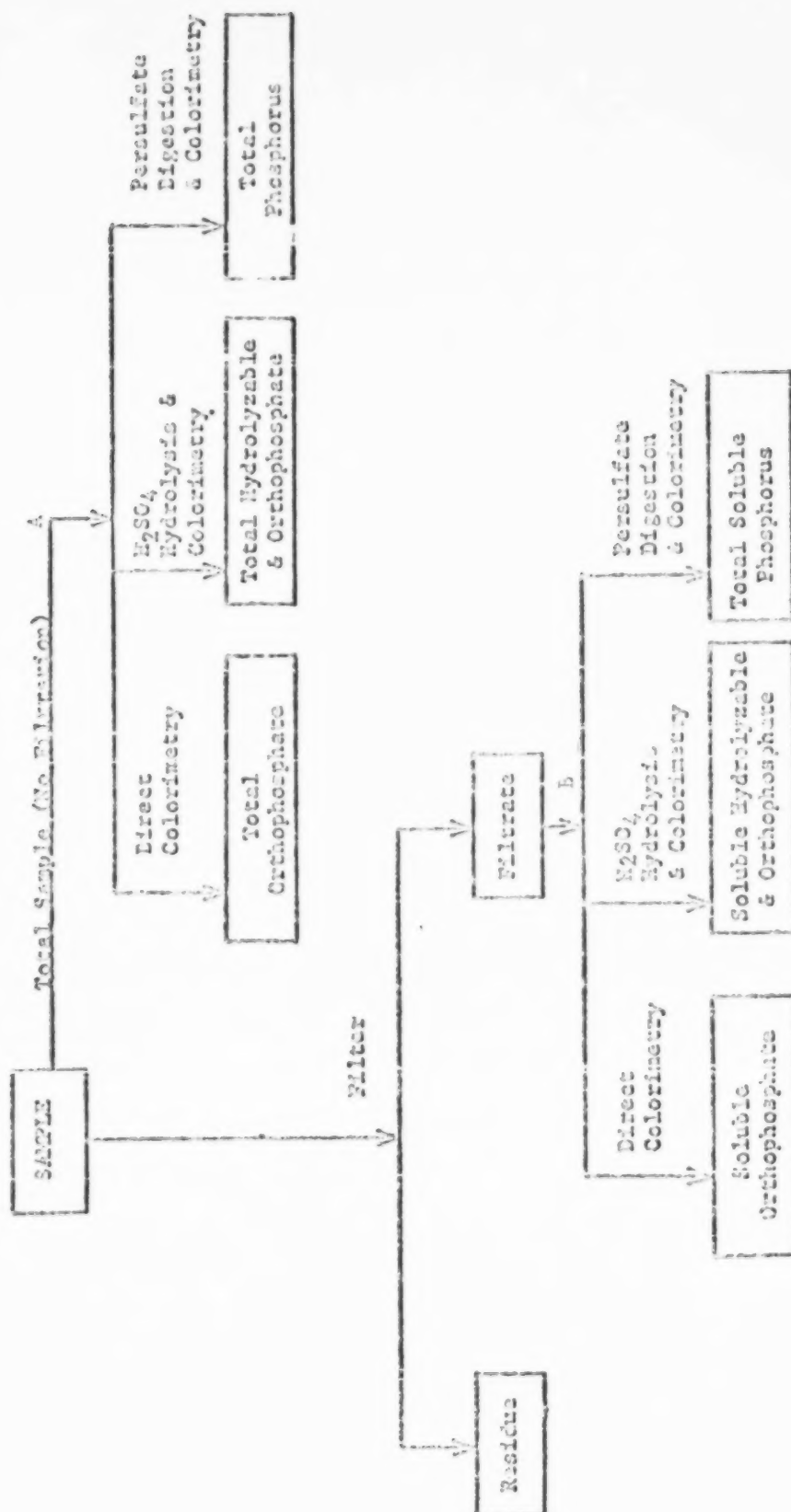
- 1) Total orthophosphate - inorganic phosphorus $[(PO_4)^{-3}]$ in the sample as measured by the direct colorimetric analysis procedure.
- 2) Total hydrolyzable phosphorus - phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined total orthophosphates. This hydrolyzable phosphorus includes polyphosphates $[(P_2O_7)^{-4}, (P_3O_{10})^{-5}, \text{etc.}]$ and some organic phosphorus.
- 3) Total organic phosphorus-phosphorus (inorganic and oxidizable organic) in the sample as measured by the persulfate digestion procedure, and minus total hydrolyzable phosphorus and orthophosphate.

B. Filtered sample

Total soluble phosphorus - all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure.

Table 1

Analytical Scheme for Differentiation of Phosphorus Forms



- 1) Soluble orthophosphate - as measured by the direct colorimetric analysis procedure.
- 2) Soluble hydrolyzable phosphorus - as measured by the sulfuric acid hydrolysis procedure and minus predetermined soluble orthophosphates.
- 3) Soluble organic phosphorus - as measured by the persulfate digestion procedure, and minus soluble hydrolyzable phosphorus and orthophosphate.

2. Sampling

2.1 Samples should be taken in a location that is the most representative of the waters in the area being sampled. If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

2.2 Sample containers may be of plastic material, such as cubitainers, or of Pyrex glass. In both cases, high iron concentrations interfere through the formation of clumps in the bottom of the sample.

3. Preservation

3.1 If the analysis cannot be performed the same day of collection, the sample should be preserved in one of the following manners (Note a):

- 3.1.1 Five ml of chloroform per liter of sample.
- 3.1.2 Acidification with sulfuric acid (used only when not measuring for all forms of phosphorus).
- 3.1.3 Quick-freezing of sample.

3.1.4 Five ml of saturated solution of mercury chloride per gallon of sample.

Note (a) -- Sufficient data is not available on these suggested preservatives to state a preference at this time.

4. Interferences

4.1 It is reported that no interference is caused by copper, iron, or silicate at concentrations many times greater than their greatest reported concentration in sea water.

4.2 The salt error for samples ranging from 5-20% salt content was found to be less than 1%.

4.3 Arsenate, in concentrations greater than found in sea water, does not interfere.

5. Apparatus

5.1 Photometer - a spectrophotometer or filter photometer suitable for measurements at 885 m μ and providing a light path for 10 cm cells.

5.2 Acid-washed glassware - all glassware used in the determination should be washed with hot HCl (1:1) and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware

should be used only for the determination of phosphorus; and, after it is used, it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with HCl (1:1) and reagents is only required occasionally. Commercial detergents containing phosphate should never be used.

6. Reagents

6.1 Sulfuric acid solution, (5N), is prepared as follows: Dilute 70 ml of conc. H_2SO_4 with distilled water to 500 ml.

6.2 Potassium antimonyl tartrate solution is prepared as follows: Weigh 1.372 g of potassium antimonyl tartrate $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$ in 400 ml of distilled water, warming if necessary. Dilute to 500 ml. The solution is stable for many months if stored in a dark, glass bottle at 4°C .

6.3 Ammonium molybdate solution is prepared as follows: Dissolve 20 g of ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 500 ml of distilled water. Store in a plastic bottle at 4°C .

6.4 Ascorbic acid is prepared as follows: Dissolve 8.8 g of ascorbic acid $\text{C}_6\text{H}_8\text{O}_6$ in 500 ml of distilled water. The solution is stable for about a week if stored at 4°C .

6.5 Combined reagent is prepared as follows: Mix thoroughly together 250 ml of 6.1, 25 ml of 6.2, 75 ml of 6.3, and 150 ml of 6.4. . . .

(total volume about 500 ml). Prepare fresh before use; do not store more than one day (if refrigerated). Mix after addition of each reagent.

6.6 Primary phosphorus standard is prepared as follows: Dissolve in distilled water .2197 g of potassium dihydrogen phosphate, KH_2PO_4 , which has been dried in an oven at 105°C . Dilute the solution to 1000 ml; 1.00 ml equals 0.05 mg P.

6.7 Working phosphorus standard is prepared as follows: Dilute 10.0 ml of stock phosphorus solution to 1000 ml with distilled water; 1.00 ml equals 0.5 μg P. Other strengths of working standards may also be made up from the stock.

6.8 Strong-acid solution is prepared as follows: Slowly add 310 ml conc. H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 liter.

6.9 Potassium persulfate - analytical reagent grade.

7. Procedure

7.1 Preparation of calibration

7.1.1 Standard curve: Prepare a series of at least nine standard phosphorus solutions to cover the range from 0 to 0.50 P mg/l. Make up the standards by diluting suitable volumes of working phosphorus solution to 100 ml with distilled water. The following dilutions are suggested:

<u>ml of working phosphorus standard</u>	<u>conc., mg/l</u>
0	0.00
1.0	0.005
3.0	0.015
5.0	0.025
10.0	0.05
20.0	0.10
30.0	0.15
40.0	0.20
50.0	0.25

- 7.1.2 Develop color in the series of standards and blank of distilled water as directed in 7.5.1 and 7.5.2 below. Procedure: Measure the color absorbance of each standard at 885 m μ with a spectrophotometer, using the reagent blank as the reference solution. Plot the absorbance values as ordinates and the corresponding phosphorus concentrations as abscissas.
- 7.1.3 Process standards and blank exactly as the samples. Run at least a blank, two standards, and a sample spiked with the standards with each series of samples. If the standards do not agree within $\pm 2\%$ of the true value, prepare a new calibration curve. The absorbances of the standards, samples, and spiked samples are used to calculate the recovery and conc. of std./absorb. std.

7.2 Total phosphorus

- 7.2.1 Add 1 ml of strong-acid solution to a 50 ml unfiltered sample in a 125 ml Erlenmeyer flask with 3 boiling beads.
- 7.2.2 Add 0.4 g of potassium persulfate.
- 7.2.3 Boil gently on a preheated hot plate for approximately 30-40 minutes keeping the volume between 20 and 50 ml. Do not allow the sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi). Filter if turbid.
- 7.2.4 Cool and adjust pH to 6-7 with (1:1) NH_4OH .
- 7.2.5 Bring to 100 ml volume.
- 7.2.6 The sample is now ready for determination of phosphorus as outlined in 7.5.2, orthophosphate.

7.3 Total hydrolyzable phosphorus

- 7.3.1 Add 1 ml of strong-acid solution to a 50 ml unfiltered sample in a 125 ml Erlenmeyer flask.
- 7.3.2 Boil gently on a preheated hot plate for approximately 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow the sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi). Filter if turbid.
- 7.3.3 Adjust the pH to 6-7 with (1:1) NH_4OH .
- 7.3.4 Bring to 100 ml volume.
- 7.3.5 The sample is now ready for determination of phosphorus as outlined in 7.5.2, orthophosphate.

7.4 Total soluble phosphorus

7.4.1 Filter appropriate volume of the sample (up to 100 ml) through a .45 μ Millipore filter.

7.4.2 Treat the sample the same as total phosphorus, carrying the blank, standards, samples, and the samples plus standard spikes throughout the procedure.

7.5 Soluble orthophosphate

7.5.1 Filter an appropriate volume of sample (up to 100 ml) through a .45 μ Millipore filter. Add 1 drop of phenolphthalein indicator to the sample. If a red color develops, add strong-acid solution, drop by drop to just discharge the color. Bring to 100 ml volume if necessary. Carry water blank, 2 standards, and samples spiked with standards through the procedure.

7.5.2 Add 16 ml of combined reagent and .2 g NaCl* to the sample and mix thoroughly. After a minimum of ten minutes, measure the color absorbance of each sample at 885 μ with a spectrophotometer, using the reagent blank as the reference solution. The color is stable for at least 2 hours.

8. Calculation

8.1 Obtain the concentration value of the sample by multiplying the corrected absorbance by the dilution factor and the conc. of std./abs.std.

Report the results as P, mg/l.

mg/l of phosphorus =

$$\frac{(\text{abs. sample} - \text{abs. blank}) \times \frac{(\text{conc. of std.}) \times (\text{dilution factor}) \times 1000}{\text{abs.}}}{\text{ml of sample}}$$

Note -- Dilution factor is one if no dilutions are made on the sample.

- * Mercury preserved samples of low salt content form precipitate when color reagent is added. Addition of NaCl apparently prevents this formation.

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1. Scope

1.1 Silica in solution as silicic acid or silicate reacts with ammonium in acidic solution to form molybdosilicic acid complex. The complex is yellow in its oxidized form and is reduced by sodium sulfite to the molybdate blue color.

1.2 Soluble unreactive silica can be digested with sodium bicarbonate.

2. Sampling

2.1 Samples should be collected in plastic containers. The use of glassware should be avoided as much as possible, especially glassware that has been in contact with alkaline solutions. When it is necessary to use glassware, use only clean, unetched, borosilicate glass.

3. Preservation

3.1 To retard biological activity in the sample, preserve the sample with 10 ml/l of 40 mg Hg^{++} /ml as HgCl_2 .

4. Interferences

4.1 Arsenic, germanium, phosphorus, aluminum, zinc, iron, and calcium interfere. Adjustment of pH and reagents eliminates and controls the effect of these interfering substances.

5. Apparatus

5.1 Spectrophotometer or photometer with absorbance measured at 650 m μ or 815 m μ with a cell path of 10 mm to 100 mm.

6. Reagents

6.1 Standard silica solution is prepared as follows: Dissolve 4.73 g $\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$ in water in a plastic beaker and dilute to 900 ml in a plastic

graduate. Withdraw 3-100 ml portions for gravimetric analysis. From the triplicate gravimetric calculate the concentration of the silica.

6.2 Ammonium molybdate solution is prepared as follows: Dissolve 100 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in silica-free distilled water, buffer with silica-free ammonia to pH 7-8, and dilute to 1 liter.

6.3 Hydrochloric acid solution is prepared as follows: Mix one volume conc. HCl with one volume of distilled silica-free water.

6.4 Aminonaphthol sulfonic acid solution is prepared as follows: Dissolve 0.5 g of 1-amino-2-naphthol-4-sulfonic acid in 50 ml of a solution containing 1 g Na_2SO_3 . Add the solution to 100 ml of a solution containing 30 g of NaHSO_3 . Dilute to 200 ml, filter if necessary, and store in a plastic bottle.

6.5 Oxalic acid solution is prepared as follows: Dissolve 10 g of oxalic acid in distilled water and dilute to 100 ml.

7 Procedure

7.1 In a 125 ml Erlenmeyer flask add 50 ml of sample or an aliquot diluted to 50 ml. To the sample add the following in rapid succession: 1.0 ml of HCl, 2.0 ml $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ solution, mix, and allow reaction to develop for 5 to 6 minutes.

7.2 Add 1.5 ml of oxalic acid solution and mix. Allow reaction to develop for at least 2 minutes, but not more than 15 minutes, add 2.0 ml of (6.4) ANSA solution, and mix.

7.3 After color development time of at least 5 minutes, measure the absorbance of sample, the blanks, and at least three standards. The

color of the molybdosilicic acid complex is stable for 12 hours.

8. Calculation

8.1

$$\text{mg/l SiO}_2 = \frac{(A_c - A_b) \frac{c}{A_s} \times D_f \times 1000}{\text{ml}_c}$$

in which:

A_c = Absorbance of samples

A_b = Absorbance of blank

A_s = Absorbance of standards

c = Concentration of standards

ml_c = ml sample

D_f = Dilution factor

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Automated Method

1. Scope

1.1 Ammonium molybdate at approximately pH 1.2 reacts with silica and also with any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicic acid. The yellow molybdosilicic acid is reduced by aminonaphthol sulfonic acid to heteropoly blue. Absorption is measured at a wave length of 660 or 815 m μ .

2. Sampling

2.1 See silica method

3. Preservation

3.1 See silica method

4. Interferences

4.1 See silica method

5. Apparatus

5.1 Technicon Autoanalyzer, consisting of sampler II, proportioning pump, two time delay coils, colorimeter with red sensitive photocell, recorder, and plastic sample cups.

5.2 The manifold is set up according to the flow diagram. The sample stream segmented with air is joined by mixed streams of ammonium molybdate and sulfuric acid. The combined streams are mixed and then passed through a time delay coil. The stream is then segmented with oxalic acid, mixed, segmented with aminonaphthol sulfonic acid, mixed, and passed into a time delay coil to give further time for reduction to take place. The stream

is then pumped through a colorimeter where the optical density is measured and recorded on the recorder for a permanent record.

6. Reagents

6.1 Ammonium molybdate solution is prepared as follows: Dissolve 20 g of ammonium molybdate in distilled water and dilute to 1000 ml.

6.2 Oxalic acid solution is prepared as follows: Dissolve 50.0 g of oxalic acid in distilled water and dilute to 1000 ml.

6.3 Sulfuric acid solution is prepared as follows: Add 5.6 ml of concentrated sulfuric acid in distilled water and dilute to 1000 ml.

6.4 Aminonaphthol sulfonic acid, (1-amino-2-naphthol-4-sulfonic acid), is prepared as follows: Dissolve 150 g of NaHSO_3 and 5.00 g of Na_2SO_3 in 800 ml of distilled water. Heat the solution to 50°C and add 2.5 g of ANSA, stirring until dissolved. Dilute to 1250 ml and filter. Store in amber bottles and refrigerate.

6.4.1 Aminonaphthol sulfonic acid working solution is prepared as follows: Dilute 100 ml of stock solution to one liter. Filter if necessary.

6.5 Silica stock standard solution is prepared as follows: Dissolve 4.730 g of sodium metasilicate nonahydrate C.P. in distilled water and dilute to 1000 ml. Test this standard according to Method A in Standard Methods, 12th Edition, pages 259-260 and adjust standard to 1.000 mg SiO_2 per ml. Label "Stock Standard Solution of Silica" and store in a plastic bottle.

6.5.1 Working standards are prepared as follows: Dilute stock solution with distilled water to the levels required for the samples analyzed. The manifold described will require 5, 10, 25, and 50 mg/l standards.

7. Procedure

7.1 Set up sampler with rate of 20 samples per hour.

7.2 Set up manifold as per diagram.

8. Calculation

8.1 Description of computational procedures is as follows:

A = Absorbance from peak and base line heights:

8.1.1 In all cases, compute absorbance (or a number directly proportional to it) as,

$$A = -\log \left(1 - \frac{p}{w-b} \right) \quad \text{where:}$$

p = peak height in cm (a function of concentration)

b = base line height in cm

w = chart width in cm (28)

8.2 Slope of regression line, $A = f(C)$:

$$S = \frac{\sum_{i=1}^n A_i C_i}{\sum_{i=1}^n C_i^2}$$

where: n = number of standards such that

$$C_{\text{sample}} = \frac{A_{\text{sample}}}{S}$$

C = concentration of standards

8.3 Calculation of sample concentration:

$$C_d = \frac{A}{S}$$

$$C_o = C_d f$$

Where: C_d = concentration of diluted sample

C_o = concentration of original sample

October 1

D

Technicon Silica III F

CONCENTRATION RANGE

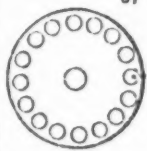
0.1 to 50 mg/l as SiO₂

TYPE SAMPLE River Water, Lake Water, and Filtered Sewerage

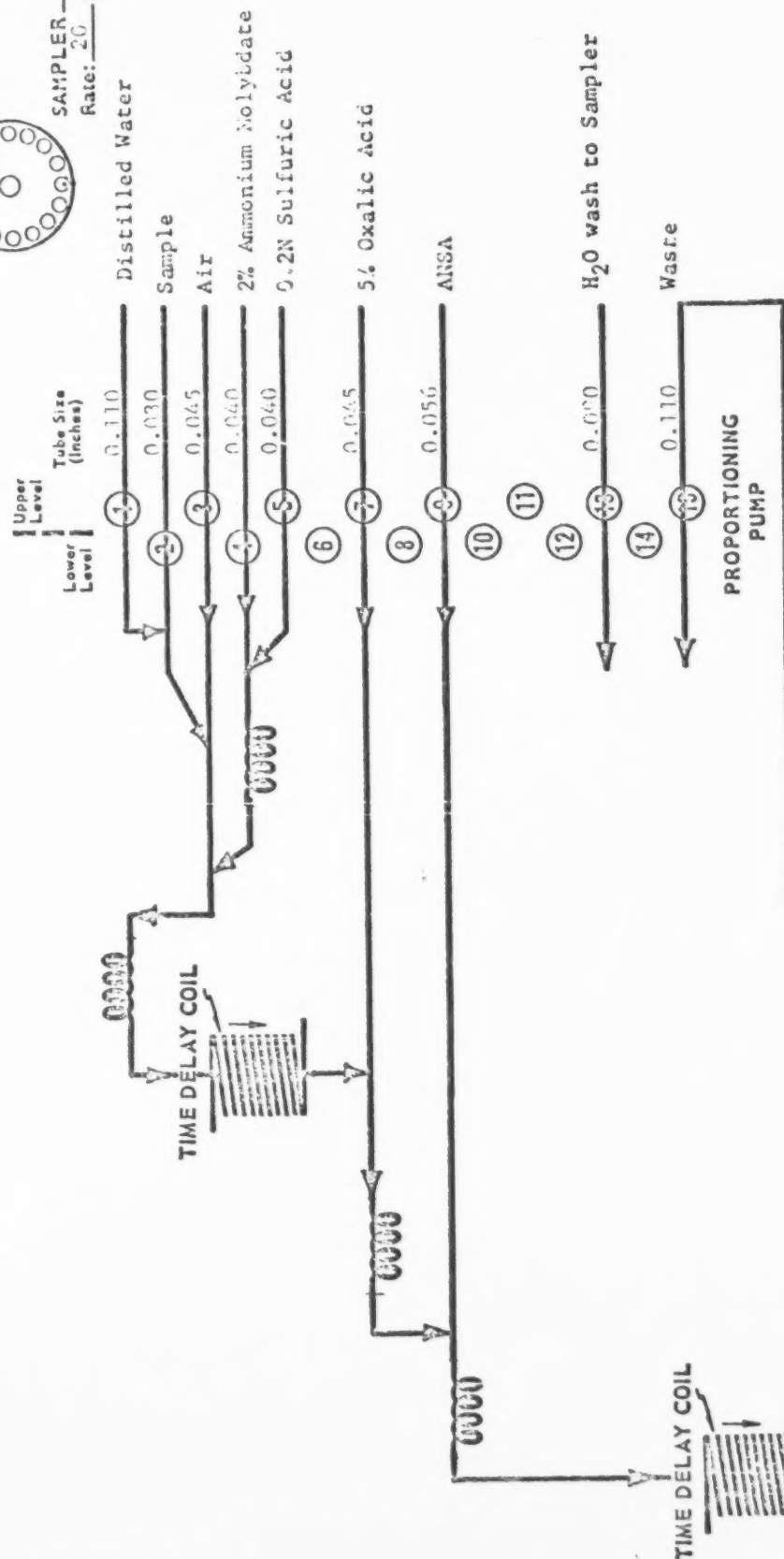
REFERENCE Technicon Silica III F (Water Analysis)

SIGNATURE

AFFILIATION



SAMPLER 17
Rate: 20 per ho



REFERENCES

Garrett, H.E., and Walker, A. J. The spectrophotometric determination of silicic acid in dilute solution. *Analyst*, 89:642-650. 1964.

Morrison, I. R., and Wilson, A. L. The absorptiometric determination of silicon in water. *Analyst*, 88:104, 446-455. 1963.

Scholes, P. H., and Thulbourne, C. Automatic procedures for the colorimetric determination of phosphorus; manganese, and silicon in steel. *Analyst*, 89:466-474. 1964.

Webber, H. M., and Wilson, A. L. The absorptiometric determination of silicon in water. *Analyst*, 89:632-641. 1964.

1. Scope

1.1 This method is applicable to the measurement of the electrical conductivity of water.

1.2 The conductivity is measured at 25°C, avoiding the use of a temperature correction and thus eliminating a major source of error in the measurement.

1.3 For the purposes of this method, the cell constant of the conductivity cell used shall be known within ± 1 percent. The manufacturer's certification of the cell constant within this accuracy shall be considered satisfactory. If the conductivity cell has been in service subsequent to this certification, it shall be rechecked by the manufacturer, or in the laboratory.

2. Sampling

2.1 Samples shall be collected to minimize absorption of gases or loss of gases.

3. Preservation

3.1 No preservative will be used. Samples should be kept tightly stoppered to prevent evaporation and analyzed as rapidly as possible.

4. Apparatus

4.1 The measuring circuit instrument may be battery, or a-c or d-c line operated. In any case the conducting cell shall be energized with an alternating current at an approximately constant frequency within the range of 25 to 3,000 cycles per second. The instrument may be manually operated Wheatstone Bridge, a deflection meter, or a self balancing

recorder or indicator. Calibration may be in conductance or resistance units. If manually operated the null-balanced detector may be a galvanometer headphone or electron ray tube of a sensitivity commensurable with the required precision of measurement.

4.2 Conductivity cells may be flow, in line, dip type, or pipette type cells for conductivity greater than 10 micromhos per cm. Cell constants vary from 0.01 to 10 or greater depending upon the conductivity of the water measured. A cell shall be chosen which will give a cell resistance in the range of 500 to 10,000 ohms.

4.3 Water bath capable of maintaining a temperature at $25^{\circ}\text{C} \pm .1^{\circ}$.

5. Reagents

5.1 Potassium chloride (KCL) is prepared as follows: Dry potassium chloride at 110°C and then heat at approximately 500°C to a constant weight. Equivalent weight KCL = 74.555.

5.1.1 Electrical Conductivity Values at 25°C

for

Potassium Chloride Reference Solutions

Normality of Solution	Weight of KCL grams/liter	Electrical Conductivity micromhos/cm
0.1	7.4555	12,896.
0.01	.7456	1,412.7
0.001	.0746	147.

6. Procedure

6.1 Place two flasks each of sample and standard KCL in a 25° water bath.

6.2 Allow 30 minutes to reach thermal equilibrium.

6.3 Rinse the conductivity cell in one of the standard KCL solutions and read the conductivity of the other.

6.4 Rinse the conductivity cell in one of the flasks containing sample; then, transfer the cell to the second flask of the same sample and read conductivity.

7. Calculation

7.1 Divide the specific conductance of the standard KCL solution by the conductance reading. Multiply this factor by the conductance reading on a sample to obtain specific conductance.

7.2 Report specific conductance in micromhos/cm at 25°C.

7.3 Example calculation:

M of KCL = 0.001

Specific conductance of 0.001N KCL = 147.0

Conductance reading of KCL = 1500

Conductance reading of sample = 450

Cell constant = $\frac{147}{1500} = 0.098$

Specific conductance of sample = $(0.098) (450) = 44$

REFERENCES

Industrial Water; Atmospheric Analysis, Part 23, p. 179-186,
Standard Methods of Test for Electrical Conductivity of Industrial Water
and Industrial Waste Water, ASTM D1125-64. American Society for Testing
and Materials, Philadelphia, Pennsylvania. 1967.

Total Residue

1. Scope

1.1 Total solids are soluble and insoluble residues as they occur in water. The total solids do not measure the total weight of constituents as they occur in water. Dissolved gases, volatile components, and decomposition products from heating are not measured by the total solids determination.

2. Sampling

2.1 Collect the samples in clean, rinsed containers of glass or plastic. Use plastic or glass that will not affect the analysis.

3. Preservation

3.1 Keep the container tightly stoppered to prevent evaporation.

4. Apparatus

4.1 Evaporating dishes.

4.1.1 Platinum - platinum dishes are the most desirable for residue determination. The high cost of platinumware limits its use in routine work.

4.1.2 Porcelain - porcelain dishes of 50 ml, 75 ml, and 100 ml size when cured properly will give satisfactory service.

4.1.3 Borosilicate glass - the borosilicate glass including the high silica borosilicate variety will be affected by highly mineralized waters.

4.2 Steam bath

4.3 Drying oven - a thermostatic control device preferably with air circulation for uniform temperature conditions.

4.4 Desiccator.

4.5 Analytical balance.

5. Procedure

5.1 The clean, dried, and weighed dish is placed on a steam bath. Add a volume of a well mixed sample to the dish which will yield a residue of 25 to 250 mg.

5.2 Apply heat to steam bath to evaporate water.

5.3 Remove dish from steam bath, place into drying oven at 105°C, and dry to constant weight. Cool the dish in a desiccator before making weighings.

6. Calculation

6.1 The calculation is carried out as follows:

Total solids =

$$\frac{\text{weight of dish in gms} + \text{residue} - \text{weight of dish in gms}}{\text{ml of sample}} \times 10^6$$

REFERENCES

Horowitz, W. Official Method of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington, D. C., 10th Edition, 1965.

Slonim, A. R. Analysis of total solids by the vacuum rotary evaporation method. Journal Water Pollution Control Federation, 39(5):846-849. 1967.

Slonim, A. R., and Crawley, F.F. Precise determination of total solids in water. Journal Water Pollution Control Federation, 38(10):1609-1613. 1966

Nonfiltrable Residue

1. Scope

1.1 The insoluble residues in water are identified as suspended solids and nonfiltrable residues. The insoluble residue may be organic or inorganic particulate matter, or finely divided crystals, plates, needles, or gels. The determination of insoluble residue consists of removing the insoluble matter by entrapment onto some barrier. The barrier can be an asbestos mat, a glass fiber mat, filter paper, or some other filtering material.

2. Sampling

2.1 Collect the samples in clean, rinsed containers of glass or plastic. Use plastic or glass that will not affect the analysis of the samples.

3. Preservation

3.1 Keep the containers tightly stoppered to prevent evaporation of the water.

4. Apparatus

4.1 Gooch crucible - porcelain.

4.2 Filtering apparatus - to hold the Gooch crucible with an appropriate connection for vacuum application and filtrate removal.

4.3 Drying oven - a thermostatic control device preferably with an air circulation system for uniform temperature conditions.

4.4 Desiccator.

4.5 Analytical balance

5. Reagents

5.1 Glass fiber filter circles of appropriate size to fit the Gooch crucible.

6. Procedure

6.1 It is necessary that new Gooch crucibles be ignited and seasoned, clean, and of stable weight. Ordinarily, one firing of the Gooch crucibles to 800°C for two hours will achieve weight stability. If weight stability is questioned, it may be necessary to reignite the crucibles at 600°C for suitable time intervals until stability is achieved.

6.2 Place the glass fiber filter disc in a clean Gooch crucible with the wrinkled surface of the paper facing upward. Apply suction and carefully wash the filter with 5 washings of 5 mls each. Dry the crucible in an oven at 103-105°C for one hour, allow to cool to room temperature in a desiccator before weighing, and weigh to constant weight.

6.3 Choose an appropriate sample size and filter through the Gooch crucible. The amount of residue should be between 2.5 and 100 mg. However, the characteristics of the residue may govern the filtration rate. The filtration rate should be rapid enough to prevent loss through evaporation.

6.4 After filtering the sample, dry the Gooch crucible in an oven at 103-105°C for one hour. Cool and place in a desiccator for at least 20 minutes. Weigh the dried crucible and contents to a constant final weight.

7. Calculation

7.1 The calculation is carried out as follows:

Total suspended matter =

$$\frac{(\text{gms final weight} - \text{gms initial weight}) \times 10^6}{\text{ml of sample}}$$

REFERENCES

Chanin, G., et al. Use of glass fiber filter medium in the suspended solids determination. *Sewage and Industrial Wastes*, 30(8):1062-1066. 1958.

Gratteau, J. C., and Dick, R. I. Activated sludge suspended solid determination. *Water and Sewage Works*, 115(10):468-472. 1968.

Nusbaum, I. New method for determination of suspended solids. *Sewage and Industrial Wastes*, 30(8):1066-1069. 1958.

Smith, A. L., and Greenburg, A.E. Evaluation of methods for determining suspended solids in waste water. *Journal Water Pollution Control Federation*, 35(9):940-943. 1963.

Wyckoff, B. M. Rapid solids determination using glass fiber filters. *Water and Sewage Works*, 111(6):277-280.

1. Scope

1.1 Principle: The method presented below is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Formazin polymer, which has gained acceptance as the turbidity standard reference suspension in the brewing industry, is also used as the turbidity standard reference suspension for water. It is easy to prepare and is more reproducible in its light scattering properties than the clay or turbid natural water standards previously used. The turbidity of a particular concentration of Formazin suspension is defined as 40 units. This same suspension of Formazin has an approximate turbidity of 40 units when measured on the Jackson Candle turbidimeter, therefore turbidity units based on the Formazin preparation will approximate those derived from the Jackson Candle turbidimeter but will not be identical to them.

2. Sampling

2.1 Collect samples in glass or plastic containers which will not affect sample characteristics.

3. Preservation

3.1 It is preferable to determine turbidity on the same day the sample is taken. If longer storage is unavoidable, however, samples may be stored in the dark up to 24 hours. For even longer storage,

treat 1 liter of sample with 1 g mercuric chloride. Prolonged storage before measurement is not recommended as irreversible changes in turbidity may occur. All samples should be vigorously shaken before examination.

4. Interferences

4.1 The determination of turbidity is applicable to any water sample that is free of debris and coarse sediments which settle out rapidly; although dirty glassware, the presence of air bubbles, and the effects of vibrations which disturb the surface visibility of the sample will lead to false results. The presence of "true color," that is the color of the water which is due to dissolved substances which absorb light, will cause measured turbidities to be low.

5. Apparatus

5.1 The turbidimeter shall consist of a nephelometer with light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter should be so designed that little stray light reaches the detector in the absence of turbidity and should be free from significant drift after a short warm-up period. The sensitivity of the instrument should permit detection of turbidity differences of 0.02 units or less in waters having turbidities less than 1 unit. The instrument should measure from 0-40 units turbidity. Several ranges will be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities.

5.2 The sample tubes to be used with the available instrument must be of clear, colorless glass. They should be kept scrupulously clean, both inside and out, and discarded when they become scratched or etched. They must not be handled at all where the light strikes them, but should be provided with sufficient extra length, or with a protective case, so that they may be handled. The tubes should be filled with samples and standards which have been thoroughly agitated, and sufficient time should be allowed for bubbles to escape.

5.3 Differences in physical design of turbidimeters will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, the following design criteria should be observed:

5.3.1 Light source - tungsten lamp operated at not less than 85% of rated voltage or more than rated voltage.

5.3.2 Distance traversed by incident light and scattered light within the sample tube - total not to exceed 10 cm.

5.3.3 Angle of light acceptance of the detector - centered at 90° to the incident light path and not to exceed $\pm 30^{\circ}$ from 90° .

5.3.4 Maximum turbidity to be measured - 40 units.

6. Reagents

6.1 Turbidity-free water is prepared as follows: Pass distilled water through a membrane filter having a pore size no greater than 100 μ if such

filtered water shows a lower turbidity than the distilled water. Discard the first 200 ml collected. Otherwise, use the distilled water.

6.2 Stock turbidity suspension is prepared as follows:

6.2.1 Solution (I): Dissolve 1.000 g hydrazine sulfate, $(\text{NH}_2)_2 \cdot \text{H}_2\text{SO}_4$, in distilled water and dilute to 100 ml in a volumetric flask.

6.2.2 Solution (II): Dissolve 10.00 g hexamethylenetetramine in distilled water and dilute to 100 ml in a volumetric flask.

6.2.3 In a 100 ml volumetric flask, mix 5.0 ml solution (I) with 5.0 ml solution (II). Allow to stand 24 hours at $25 \pm 3^\circ\text{C}$, then dilute to the mark and mix.

6.3 Standard turbidity suspension is prepared as follows: Dilute 10.00 ml stock turbidity suspension to 100 ml with turbidity-free water. The turbidity of this suspension is defined as 40 units.

6.4 Dilute turbidity standards as follows: Dilute portions of the standard turbidity suspension with turbidity-free water as required.

6.4.1 A new stock turbidity suspension should be prepared each month. The standard turbidity suspension and dilute turbidity standards should be prepared weekly by dilution of the stock turbidity suspension.

7. Procedure

7.1 Turbidimeter calibration: The manufacturer's operating instructions should be followed. Measure standards on the turbidimeter covering

the range of interest. If the instrument is already calibrated in standard turbidity units, this procedure will check the accuracy of the calibration scales. At least one standard should be run in each instrument range to be used. Some instruments permit adjustment of sensitivity so that scale values will correspond to turbidities. Reliance on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges is not an acceptable practice unless the turbidimeter has been shown to be free of drift on all ranges. If a pre-calibrated scale is not supplied, then calibration curves should be prepared for each range of the instrument.

7.2 Turbidities less than 40 units: Shake the sample to thoroughly disperse the solids. Wait until the air bubbles disappear; then, pour the sample into the turbidimeter tube. Read the turbidity directly from the instrument scale or from the appropriate calibration curve.

7.3 Turbidities exceeding 40 units: Dilute the sample with one or more volumes of turbidity-free water until the turbidity falls below 40 units. The turbidity of the original sample is then computed from the turbidity of the diluted sample and the dilution factor. For example, if 5 volumes of turbidity-free water were added to 1 volume of sample, and the diluted sample showed a turbidity of 30 units; then, the turbidity of the original sample was 180 units.

8. Calculation

8.1 Turbidity readings are reported in accordance with the following:

Turbidity Range Units	Record to nearest
0.0- 1.0	0.05
1. - 10.	0.1
10. - 40.	1.
40. - 100.	5.
100. - 400.	10.
400. -1000.	50.
> 1000.	100.

8.2 For comparison between samples it may be desirable to estimate more closely than is specified in the table: but, because of the uncertainties and discrepancies in turbidity measurements, it cannot be expected that two or more laboratories will duplicate results on the same sample more closely than specified.

8.3 The reproducibility of results at levels of turbidities of less than 5 units will be the subject of intensive studies. Collaborative testing at levels below 5 units will indicate the reliability of the turbidimetric determination at low levels.

REFERENCES

- Black, A. P., and Hannah, S. A. Measurement of low turbidities. Journal American Water Works Association, 57(7):901-916. 1965.
- Conley, W. R., and Pitman, R. W. Microphotometer turbidity analysis. Journal American Water Works Association, 49(1): 63-67. 1957.
- Ives, K. J., Atkin, J. R., and Thompson, R. P. Measurement of turbidity. Effluent and Water Treatment Journal, 8:342-348, 406-409. 1968.
- Palin, A. T. Photometric determination of color and turbidity of water. Water and Sewage Works, 104(11):492-495. 1957.

Platinum-Cobalt Method

1. Scope

1.1 This method is applicable to the determination of color of water and waste water for routine control purposes.

1.2 Color is measured by visual comparison of the sample to platinum-cobalt standards with one unit of color being that produced by 1 mg/l platinum, in the form of the chloroplatinate ion.

2. Sampling

2.1 Samples for the color determination should be representative and must be taken in clean glassware. The color determination should be made within a reasonable period, as biologic changes occurring in storage may affect the color.

3. Preservation

3.1 Samples may be preserved with the addition of 40 mg Hg^{++} /l (as HgCl_2) and stored at 4°C . This preservation technique is tentative pending evaluation.

4. Interferences

4.1 Even a slight turbidity interferes with the test. The recommended method for the removal of turbidity is by centrifugation.

4.2 The color is extremely pH dependent and invariably increases as the pH of the sample is raised. The pH must be measured and recorded along with the color.

5. Apparatus

5.1 Nessler tubes, matched, 50 ml, tall form.

5.2 pH meter.

6. Reagents

6.1 Dissolve 1.246 g $\cdot$ K_2PtCl_6 and 1 g $CoCl_2 \cdot 6H_2O$ (crystals) in distilled water with a 100 ml conc. HCl and dilute to 1 liter with distilled water. This stock standard has color of 500 units.

6.2 Prepare standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 70 color units by diluting stock to a 50 ml volume. Protect these standards against evaporation and contamination when not in use.

7. Procedure

7.1 Determine the color of the sample by comparing a turbidity-free sample or aliquot of sample in a 50 ml Nessler tube with standards. Make a comparison by looking vertically downward through the tubes toward a white illuminated surface placed at such an angle that light is reflected upward through the columns of liquid.

7.2 Determine pH of sample.

8. Calculation

8.1 The color is matched to a standard and a proper dilution factor is applied. Report color as follows:

Color Unit	Record Units to nearest
1-50	1
51-100	5
101-250	10
251-500	20

REFERENCES

Black, A. P., and Christman, R. F. Characteristics of colored surface water. *Journal American Water Works Association*, 55(6):753-770. 1963

Palin, A. T. Photometric determination of the color and turbidity of water. *Water and Sewage Works*, 104(11):492-495. 1957.

1. Scope

1.1 This method covers the determination of chloride ion in water. The method is applicable to water relatively low in solids. The following is a summary of the method. A dilute mercuric nitrate solution is added to a sample acidified with an acid mixed diphenylcarbazone bromophenol blue indicator. The end point of the titration is the formation of the blue-violet mercury diphenylcarbazone complex.

2. Preservation

2.1 Samples should be held in such a condition so as to prevent evaporation.

3. Interferences

3.1 The anions and cations generally found in water do not interfere.

4. Apparatus

4.1 Burette 25 or 50 ml, graduated in 0.1 ml divisions.

5. Reagents

5.1 Mercuric nitrate, standard solution - 0.0141N is prepared as follows: Dissolve 2.420 g of $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in 25 ml of water acidified with 0.25 ml of concentrated HNO_3 . Dilute the $\text{Hg}(\text{NO}_3)_2$ solution with water to 1 liter. Store away from the light in a dark bottle. Standardize against standard NaCl solution.

5.2 Sodium chloride is prepared as follows: Dry several grams of sodium chloride (NaCl) for 1 hour at 600°C . Remove from furnace and cool to 105°C in oven. Cool in desiccator. Weigh $.8241 \pm .0002$ g

of dry salt and dilute to 1 liter in chloride-free distilled water at 20°C in a volumetric flask.

5.3 Mixed indicator - acidifier is prepared as follows: Dissolve .25 g crystalline diphenylcarbazone (Eastman Kodak No. 4459S diphenylcarbazone suitable), 4.0 ml conc. HNO_3 , and .03 g xylene cyanol FF (Eastman Kodak No. T1579 suitable) in 100 ml of 95% ethyl alcohol or isopropyl alcohol. Store in a dark bottle in a refrigerator. Do not use this reagent for more than six months. This reagent can be used on samples with alkalinity levels to 150 mg/l. In samples where alkalinity is greater than 150 mg/l, acidify the sample with 0.1N HNO_3 to a pH of 2.5 ± 0.1 . Do the acidification on a separate sample using a pH meter to determine the amount of HNO_3 acid to be added. Add this quantity to a second sample which was not in contact with calomel electrode.

5.4 Nitric acid is prepared as follows: Mix 6.4 ml of conc. HNO_3 with distilled chloride-free water and dilute to 1 liter.

6. Procedure

6.1 Use a 100 ml sample or smaller aliquot diluted to 100 ml such that it will contain not more than 10 mg of chloride ion.

6.2 Add 1.0 ml of mixed indicator acidifier reagent to sample. The color of the solution should be a greenish-blue at this point. A color which is pure blue indicates pH above 3.8 and 0.1N HNO_3 must be added dropwise until a greenish-blue color is visible.

6.3 Titrate the sample with 0.0141N $\text{Hg}(\text{NO}_3)_2$ to a definite purple end point. The solution will turn from a greenish-blue to a blue a few drops from the end point.

6.4 Perform a blank determination using 100 ml of distilled chloride-free water containing 10 mg alkalinity made up by dissolving NaHCO_3 in chloride-free distilled water.

7. Calculation

7.1 Calculate the chloride ion concentration in the original sample in mg/l as follows:

$$\text{Chloride mg/l} = \frac{(\text{ml Hg(NO}_3)_2 \text{ sample} - \text{ml HgNO}_3 \text{ blank}) \times \text{Normality Hg(NO}_3)_2 \times 35,000}{\text{ml of sample}}$$

REFERENCES

Clarke, F. E. Determination of chloride in water. *Analytical Chemistry*, 22(4):553-555. 1950.

Goldman, E. New indicator for the mercurimetric chloride determination in potable water. *Analytical Chemistry*, 31(6):1127. 1959

Roberts, I. Titration of chloride ion with mercuric nitrate solutions. *Industrial and Engineering Chemistry, Analytical Edition*, 8(5): 365-367. 1936

1. Scope

1.1 This method applies to the determination of water and wastewater which is not highly colored. Calcium and magnesium ions in water are sequestered by Na_2EDTA . The indicator 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid (Calmagite) changes to a blue color when all the calcium and magnesium are sequestered.

2. Sampling

2.1 Samples should be collected in glass or plastic containers that will not release constituents which will interfere with analysis.

3. Preservation

3.1 Samples should not be preserved with a preservative. Samples should be kept in a closed container to inhibit evaporation loss.

4. Interferences

4.1 Normal constituents in water will not interfere. The cyanide inhibitor will eliminate interference of copper, zinc, lead, cobalt, nickel, and iron in concentrations greater than 0.25 mg and manganese in concentrations greater than 0.025 mg.

5. Reagents

5.1 Buffer solution is prepared as follows: Dissolve 16.9 g ammonium chloride, NH_4Cl , in 143 ml ammonium hydroxide, NH_4OH , add 1.25 g of the magnesium salt of EDTA and dilute to 250 ml with distilled water. Store in a plastic or resistant-glass container fitted with a tightly stoppered top.

5.2 Inhibitor solution is prepared as follows: Dissolve 25 g of KCN in distilled water and dilute to a liter (1 ml = .025 g KCN).

5.3 Standard EDTA titrant, (0.01M), is prepared as follows: Use analytical reagent grade disodium ethylene diamine tetraacetate dihydrate, also called (ethylenedinitrilo) tetraacetic acid disodium salt (EDTA). Weigh 3.723 g of the dry reagent, dissolve in distilled water, and dilute to 1 liter in a volumetric flask. Check the titer by standardization against standard calcium solution in the same manner that the samples are titrated. Store the solution in a plastic bottle or pyrex bottle.

5.4 Calcium standard solution, (1 ml contains 1.00 mg CaCO_3 or 0.400 mg Ca), is prepared as follows: Weigh 1.000 g anhydrous calcium carbonate, CaCO_3 , (primary standard low in magnesium) into a 500 ml Erlenmeyer flask. Place a funnel in the neck of the flask and add, a little at a time, HCl (1:1) until all the CaCO_3 has dissolved. Add 200 ml of distilled water and boil for a few minutes to expel CO_2 . Cool, transfer to a 1 liter volumetric flask, and dilute to the mark with distilled water.

6. Procedure

6.1 Use 100 ml of sample or an appropriate amount to result in a titration of less than 18 ml.

6.2 Add 2 ml ammonia buffer to sample. (pH should be 10)

6.3 Add 1 ml KCN solution to sample.

6.4 Add 0.1 g Calmagite indicator to sample.

6.5 Titrate with EDTA to a blue end point. (no pink detectable)

7. Calculation

7.1 Hardness as mg CaCO_3 per liter = $\frac{(\text{ml EDTA}) (1000) (\text{factor})}{\text{ml of sample}}$

factor = $\frac{(\text{ml standard Ca solution}) (\text{mg } \text{CaCO}_3 \text{ per ml std. Ca solution})}{\text{ml of EDTA}}$

REFERENCES

Betz, J. D., and Noll, C. A. Total hardness determination by direct colorimetric titration. Journal American Water Works Association, 42(1):49-56. 1950

Laitinen, H.A. Chemical Analysis. McGraw-Hill, New York, 1960.
p. 228-245.

Schwarzenbach, G. Complexometric Titrations. Interscience Publishers, New York, 1957.

1. Scope

1.1 Trace metals in water are analyzed by atomic absorption spectroscopy. With the exception of sodium, potassium, calcium, and magnesium, nitric acid acidified samples are concentrated to a suitable volume, maintaining a total solid concentration of less than 10,000 mg/l (1%).

2. Sampling

2.1 Samples should be collected in plastic or glass bottles. If dissolved metals are desired, filtration should be accomplished through a 0.45 μ filter in the field.

3. Preservation

3.1 Samples should be acidified with 25 ml of conc. HNO_3 per liter of sample.

4. Interferences

4.1 Phosphorus, aluminum, nitrate, and sulfate interfere with analysis of calcium and magnesium. The addition of lanthanum and hydrochloric acid eliminates the interference of phosphorus, aluminum, nitrate, and sulfate.

4.2 Total solids should not exceed 10,000 mg/l since, at concentrations of this level or above, light scattering may give a false absorbance.

5. Apparatus

5.1 Atomic absorption spectrophotometer.

5.2 Recorder.

5.3 Hollow cathode lamp.

6. Reagents

6.1 Acetylene - a good technical or commercial grade of acetylene.

6.2 Compressed air - compressed air free from oil and water.

6.3 Nitrous oxide - medical grade of nitrous oxide.

7. Procedure

7.1 In the procedure section, the instructions of the manufacturer of the atomic absorption spectrophotometer must be followed.

7.2 Under procedure, the following table will list metals, wavelength, detection limit, gas mixture, and sensitivity. The sensitivity is defined as the concentration of metal in solution that produces a 1% absorption signal.

<u>Metal</u>	<u>Wavelength</u>	<u>Detection Limit in mg/l</u>	<u>Gas Mixture</u>	<u>mg/l Sensitivity</u>
Aluminum	309.2	0.1	C ₂ H ₂ -N ₂ O	0.4
Bismuth	223.6	0.05	C ₂ H ₂ -air	0.9
Calcium	422.7	0.002	C ₂ H ₂ -air	0.045
Chromium	357.9	0.01	C ₂ H ₂ -air	0.016
Cobalt	240.7	0.002	C ₂ H ₂ -air	0.02
Copper	324.7	0.005	C ₂ H ₂ -air	0.012
Iron	248.3	0.004	C ₂ H ₂ -air	0.006
Lead	217.0	0.03	C ₂ H ₂ -air	0.065
Magnesium	285.2	0.0005	C ₂ H ₂ -air	0.008
Manganese	279.5	0.0008	C ₂ H ₂ -air	0.013
Molybdenum	313.3	0.03	C ₂ H ₂ -N ₂ O	0.6
Nickel	232.0	0.005	C ₂ H ₂ -air	0.009

Table continued:

Potassium	766.5	0.005	C ₂ H ₂ -air	0.0045
Sodium	589.6	0.001	C ₂ H ₂ -air	0.0035
Tungsten	400.9	3.0	C ₂ H ₂ -N ₂ O	20.0
Vanadium	318.4	0.2	C ₂ H ₂ -N ₂ O	1.3
Zinc	213.9	0.001	C ₂ H ₂ -air	0.0018

8. Calculation

8.1 Plot the concentration versus the readout (absorbance, peak height, % full scale, etc.). Determine con./readout, divide this by the concentration factor, and include the scale expansion, if any, to get the multiplier.

8.2 Conc. =

$$\left[\frac{\text{std. conc. (}\mu\text{g)} }{\text{liter}} \times \frac{\text{final vol.}}{\text{initial vol.}} \times \frac{\text{scale exp. factor for samples}}{\text{scale exp. factor for standards}} \right] \text{ sample readout}$$

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1. Scope

1.1 The inorganic arsenic is reduced to arsine by zinc in a Gutzeit generator. The liberated arsine reacts with $\text{AgSCSN}(\text{C}_2\text{H}_5)$ in an absorber tube after being scrubbed by a $\text{Pb}(\text{CH}_3\text{COO})_2$ solution impregnated onto glass wool to remove the sulfides. The reaction of AsH_3 with $\text{AgSCSN}(\text{C}_2\text{H}_5)$ in pyridine produces a soluble, red compound.

2. Sampling

2.1 Samples should be collected in clean and rinsed glass or plastic bottles.

3. Preservation

3.1 No preservation is required.

4. Interferences

4.1 Cobalt, nickel, iron, copper, chromium, and lead present in concentrations of 50 mg and silver and molybdenum present in concentrations of 10 mg inhibit production of arsine. Antimony reduced to stibine develops a color with maximum absorbance at 510 m μ .

5. Apparatus

5.1 Arsine generator (see Figure 1).

5.2 Spectrophotometer.

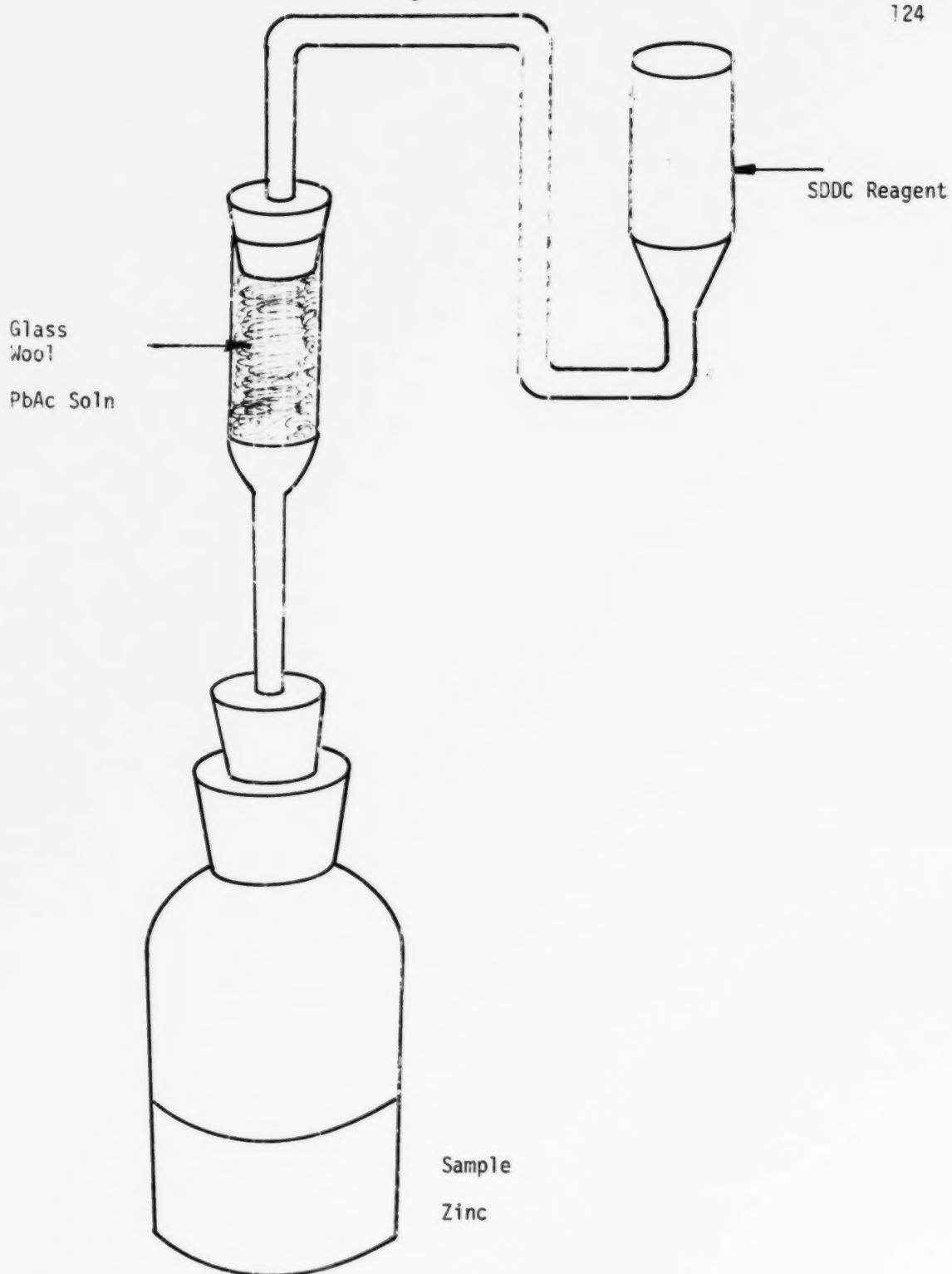
6. Reagents

6.1 Hydrochloric acid conc.

6.2 Potassium iodide solution is prepared as follows: Dissolve 15 g of KI in 100 ml of distilled water.

Figure 1

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6.3 Stannous chloride reagent is prepared as follows: Dissolve 40 g arsenic-free $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 ml conc. hydrochloric acid.

6.4 Lead acetate solution is prepared as follows: Dissolve 10 g $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ in 100 ml distilled water.

6.5 Silver diethyldithiocarbamate reagent is prepared as follows: Dissolve 1 g $\text{AgSCSN}(\text{C}_2\text{H}_5)$ in 200 ml pyridine. Store in a brown bottle.

6.6 Zinc is prepared as follows: 20-30 mesh, arsenic-free.

6.7 Arsenic stock solution, (1.00 ml = 1.00 mg As), is prepared as follows: Dissolve 1.320 g arsenic trioxide, As_2O_3 , in 10 ml distilled water containing 4 g NaOH, and dilute to 1000 ml with distilled water.

6.8 Intermediate arsenic solution, (1.00 ml = 10.0 ug As), is prepared as follows: Dilute 5.00 ml stock solution to 500 ml with distilled water.

6.9 Arsenic standard solution, (1.00 ml = 1.00 ug As), is prepared as follows: Dilute 10.00 ml intermediate solution to 100 ml with distilled water.

7. Procedure

7.1 Add 35 ml of sample into clean arsine generator bottle.

7.2 Add in order the following with thorough mixing after each addition.

7.2.1 5 ml conc. HCl (6:1).

7.2.2 2 ml KI (6:2).

7.2.3 .4 ml SnCl_2 (6:3).

7.2.4 Allow the reaction to proceed for 15 minutes to reduce the arsenic to the trivalent form.

7.3 Prepare the scrubber by impregnating glass wool with lead acetate for the removal of sulfides.

7.4 Add 4.0 ml of $\text{AgSCSN}(\text{C}_2\text{H}_5)$ (6:5) solution to the absorber tube.

7.5 Add 3 g Zn to the generator and connect the scrubber-absorber assembly immediately. All connections should be tight to prevent any gas leakage.

7.6 The reaction should continue for 30 minutes so that all the arsine can be evolved. Warm the generator slightly to release any residual arsine.

7.7 The arsine which now reacted with the $\text{AgSCSN}(\text{C}_2\text{H}_5)$ should be transferred to a spectrophotometer and the absorbance measured at 540 m μ .

7.8 Standards used to prepare a calibration graph should be handled in the same manner as samples going through reduction, scrubbing, and reaction in the absorber. Prepare standards to give at least five points on the graph.

8. Calculation

8.1 Calculation is carried out as follows:

$\mu\text{g/l As} =$

$$\frac{(\text{Absorb. of sample} - \text{Absorb. of blank}) \times \frac{\mu\text{g stand.}}{\text{Absorb. stand.}} \times 1000}{\text{ml of sample}}$$

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Stratton, G., and Whitehead, H. C. Colorimetric determination of arsenic in water with silver diethyldithiocarbamate. Journal American Water Works Association, 54(7):861-864. 1962.

1. Scope

1.1 Boron reacts with curcumin in the presence of acid just before the reaction mixture has completely evaporated to produce a red-colored reaction product of rosocyanine. The rosocyanine is dissolved in an organic solvent and is determined colorimetrically at a wavelength of 540 mμ.

2. Sampling

2.1 Samples should be collected in plastic containers free of boron, or alkali resistant, boron-free glassware.

3. Preservation

3.1 No preservation is required.

4. Interferences

4.1 Iron, magnesium, and potassium in the absence of sodium, if found at levels 100 times greater than normally present in sea water, will interfere slightly.

5. Apparatus

5.1 Spectrophotometer.

5.2 Evaporating dishes - porcelain dishes rinsed in distilled water and used only in boron analysis.

5.3 Water bath - circulating water bath with thermostatic setting for $55^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

5.4 Volumetric flasks, glass stoppered with a 25 ml capacity.

6. Reagents

6.1 Boron stock solution is prepared as follows: Dissolve 0.5715 g of analytical reagent grade H_3BO_3 in distilled water and dilute to a liter. This solution contains 100 μg of B per ml.

6.1.1 Boron standard working solution is prepared as follows:

Dilute 10 ml of (6.1) to a liter. This solution contains 1 μg B per ml.

6.2 Curcumin reagent is prepared as follows: Dissolve 0.040 g of finely ground curcumin and 5.0 g oxalic acid in 80 ml of 95% ethanol. Add 4.2 ml conc. HCl and dilute to 100 ml with ethanol. This reagent is stable for several days if refrigerated.

7. Procedure

7.1 Transfer by pipette 1.0 ml of sample containing 0.1-1.0 μg B to a porcelain evaporating dish.

7.2 Add 4.0 ml of curcumin reagent (6.2) and swirl the dish gently to mix contents thoroughly.

7.3 Float the dishes on a water bath set at $55^\circ C \pm 2^\circ C$ and evaporate the contents to complete dryness.

7.4 Remove the dish from the water bath as soon as the contents appear dry and odor of HCl is gone.

7.5 Allow the dishes to cool to room temperature, add 10 ml of 95% ethanol, and stir and mix to dissolve the red-colored reaction product.

7.6 Wash the contents of each dish into a 25 ml volumetric flask using 95% ethanol. Bring up to volume.

7.7 Measure the absorbance at the wavelength of 540 mμ.

7.8 Treat blank and at least three standards in the same manner as samples.

7.9 Run calibration data for at least five standards to develop a calibration graph or reciprocal of absorbance per μg of S.

8. Calculation

8.1 Calculation is carried out as follows:

μg/l B =

$$\frac{(\text{Absorb. of sample} - \text{Absorb. of blank}) \times \frac{\mu\text{g conc.}}{\text{Absorb.}} \times 1000}{\text{ml of sample}}$$

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Hayes, M. R., and Metcalfe, J. The boron-curcumin complex in the determination of trace amounts of boron. *Analyst*, 87:956-969. 1962.

Lishka, R. J. Comparison of analytical procedures for boron. *Journal American Water Works Association*, 53(12):1517-1522. 1961.

1. Scope

1.1 Chlorophyll is a measure of plant pigments and is reported in S.P.U. "specific plant unit or in mg/m^3 ." The limit of detection of plant pigments in water cannot be simply stated because an unlimited volume of water may be filtered for analysis. In practice, however, the volume filtered will rarely exceed 10 liters.

1.2 The larger zooplanktons are removed by straining a sample of water through a nylon net of about 300 μ mesh size and then the phytoplanktons are filtered onto a Millipore AA filter or a glass filter. Pigments are extracted from the algae cells for examination spectrophotometrically (see Note a).

2. Sampling

2.1 Adequate sampling of the euphotic zone or detrital layers for phytoplankton is a subject which is outside the scope of the present method. Once obtained, the final sample (generally 500 ml to 5 liters in volume) is filtered through a small piece of clean 0.3 mm mesh nylon netting to remove the larger zooplankton. The required volume of this filtrate should be measured by a polyethylene measuring cylinder into a polyethylene bottle.

3. Preservation

3.1 Two or three drops (ca. 0.1-0.2 ml) of magnesium carbonate suspension are added to the sample (see Note b). The sample may then be stored in a cool dark place for a maximum of about 8 hours. It is

desirable, however, that samples be filtered through a membrane filter at the time of collection.

3.2 Membrane filters can be stored by folding them in half (with the plankton innermost) and storing them in the dark in a desiccator frozen to -20°C but only for a few weeks. This procedure almost always leads to low results and makes the extraction of chlorophyll more difficult. Filters should be extracted without delay, if at all possible.

4. Apparatus

4.1 Millipore filtration equipment designed to hold 47 mm diameter membrane filters.

4.2 One 300 ml polyethylene wash bottle.

4.3 Stoppered graduated centrifuge tubes of 15 ml capacity having both glass and polyethylene stoppers.

4.4 "Small volume" spectrophotometer cells having a path-length of 10 cm but holding 10 ml or less of solution.

5. Reagents

5.1 Reagents: Distill reagent grade acetone over about 1% of its weight each of anhydrous sodium carbonate and anhydrous sodium sulphite. Collect the fraction boiling at a constant temperature near 56.5°C (uncorrected). One hundred ml of water is pipetted into a liter volumetric flask and acetone added to make the volume exactly 1000 ml. The redistilled acetone should be stored in a tightly stoppered dark glass bottle and the 90% reagent prepared in moderately small amounts (say a liter at a time)

for use. This reagent is conveniently dispensed from a polyethylene wash bottle which should be kept nearly full.

5.2 Magnesium Carbonate Suspension is prepared as follows: Add approximately 1 g of finely powdered magnesium carbonate of analytical reagent quality to 100 ml of distilled water in a stoppered Erlenmeyer flask. Shake vigorously to suspend the powder immediately before use.

6. Procedure

6.1 Invert the polyethylene bottle containing the sample into the funnel of the Millipore filter equipment fitted with either a 47 mm diameter Millipore AA filter or a 4.5 cm Whatman GF/C glass filter paper (see Note a). The bottle need not be rinsed but the contents should be shaken vigorously, before filtration is commenced. If not added previously, introduce about 1 ml of magnesium carbonate suspension to the last few hundred milliliters of sample being filtered (see Note b).

6.2 Drain the filter thoroughly under suction before removing it from the filtration equipment; and, if a Millipore filter is used, trim away the peripheral excess of unstained membrane with clean scissors (see Note c). Store the filter if necessary but if possible extract the pigment without delay.

6.3 Place the filter in a 15 ml stoppered graduated centrifuge tube.

6.3.1 If a Millipore filter was used, add approximately 8 ml of 90% acetone, stopper, and dissolve the filter by shaking the tube vigorously. If a glass paper was used, add approximately 10 ml of 90% acetone, stopper and disperse

and disintegrate the paper by shaking the tube vigorously (see Note d). Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20 hours (see Notes e and f). It is good practice to shake the tubes vigorously once more after they have been an hour or two in the refrigerator.

6.4 Remove tubes from the refrigerator and let them warm up in the dark nearly to room temperature. Add 90% acetone to make the extracts from Millipore filters exactly 10.0 ml and those from glass filters exactly 12.0 ml (see Note g). Centrifuge the content of the tubes for 5-10 minutes (see Note h) having replaced the glass stoppers on the centrifuge tubes with plastic stoppers to prevent breakage during centrifugation.

6.5 Decant the clear supernatant liquid into a 10 cm path-length spectrophotometer cell designed to hold 10 ml or less of liquid. In the event of absorbance values exceeding about 1.3, the measurements described below should be made with 2.5 cm or 1 cm cells and the absorbance values multiplied by 4 or 10, respectively, to normalize them to the values expected with a 10 cm cell. If 12 ml of acetone is used with glass papers, multiply the absorbance value by 1.2 to normalize them to the values expected from 10 ml of extract.

6.6 Without delay measure the absorbance of the solution against a cell containing 90% acetone (see Note i) at 750, 665, 645, and 630 m μ (see Note j). Record the absorbance values to the nearest 0.001 unit in the

range 0-0.4 and the nearest 0.005 for absorbance exceeding about 0.4. Correct at each wavelength by the procedure described in "6.8" below.

6.7 As the precise values of comparatively small extinctions have to be measured, corrections for all optical inequalities become important. Fill both spectrophotometer cells with 90% acetone and find the cell-to-cell blank of the sample cell against the reference cell at all wavelengths used in the method. Correct all extinction values by this cell-to-cell blank which may amount to 0.01 or more.

6.8 Turbidity Blanks

6.8.1 If glass papers are used there should be only a very small blank. This is measured by the spectrophotometer reading at 750 m μ where there is known to be no absorption of light from pigments. We have sometimes found a small negative blank for reasons which are not clear. In any case the value, positive or negative, should not exceed about 0.002 and may be corrected for cell-to-cell blank and used for the absorbance at all wavelengths.

6.8.2 A certain amount of colloidal material remains after the solution of an AA Millipore filter, even after centrifugation. The absorbance from this material depends on the wavelength of light used, increasing at shorter wavelengths due to light scattering effects.

6.8.3 The absorbance at 750 m μ is corrected for any cell-to-cell blank at this wavelength and the resulting absorbance (A_b)

is multiplied by a factor f to give the turbidity blank extinction to be used with spectrophotometer readings at other wavelengths.

Total blank correction = cell-to-cell blank + ($f \times A_b$),

where f has the values shown below:

Wavelength $m\mu$	f
665	1
645	1
630	1
510	2

6.8.4 It must be stressed that these values for f are very approximated. If a good correction is required, A_b must not exceed about 0.02.

7. Calculation

7.1 Calculate the concentration of pigments in sea water from the equation:

$$\text{mg pigment/m}^3 = \frac{C}{V}$$

where C is a value obtained from the following equations and V is the volume of sea water filtered in liters. When the Parsons-Strickland equations are used, values for chlorophylls a and b, will be in mg/m^3 .

7.2 Formulae: P.S. = Parsons and Strickland. A stands for the absorbance values in $m\mu$ units at wavelengths indicated by the subscripts measured in 10 cm cells after correcting for a blank as described in 6.8 above.

P.S. C(chlorophyll a) = 11.6 A665 - 1.31 A645 - 0.14 A630

P.S. C(chlorophyll b) = 20.7 A645 - 4.34 A665 - 4.42 A630

Note (a) Millipore filters have the advantage that they dissolve in acetone completely, give no complications at the centrifugation stage, and no particular precautions are necessary during filtration. These filters are expensive. Glass filters are cheaper and their use results in practically no blank. They are recommended if a cell grinding step is required to give better extraction (see Note d). However, care must be taken when filtering samples through the comparatively coarse glass filters and trouble is experienced at the centrifugation stage. A manostat (there are several cheap commercial laboratory units based on the cartesian diver) must be used with glass papers to ensure that the suction never exceeds one-quarter to one-third of an atmosphere or else pigment may pass through the filters.

Note (b) The magnesium carbonate is added at this stage to ensure that the phytoplankton chlorophyll is prevented from becoming acid with the resulting decomposition to give pheophytin pigments. We have some doubts of the efficacy of such an addition compared with, say, the addition of a completely soluble organic base but the use of magnesium carbonate is established practice and doubtless has some value as a precautionary measure. Care should be taken to see that the Millipore filtration equipment, centrifuge tubes, and the spectrophotometer cells are kept free from acid and that the filter is not touched with acidic fingers.

Note (c) The troublesome blank, measured at 750 m μ found with Millipore filters is caused almost entirely by the salt left in the filter at this stage which subsequently "salts out" membrane material from the acetone. The blank can be greatly reduced if filters are sucked dry of water very thoroughly at this stage and as much as possible of the unwanted peripheral filter is cut away.

Note (d) If poor extraction is anticipated, use a glass filter and after filtration, push this to the bottom of a "Potter" type grinder holding about 20 ml. Following Yentsch and Menzel (Deep-Sea Research, 10: 221, 1963) we have used the Arthur H. Thomas grinder No. 4288-B fitted to an ordinary laboratory stirring motor. About 2 ml of 90% acetone should be added and the grinder run for 1-2 minutes in subdued light. The tube should be pushed up and down the pestle during the extraction; but, for much of the time, the pestle should be hard against the bottom of the tube. After use, the pestle is rinsed into the tube with a few milliliters of 90% acetone which is also used to transfer the content

of the grinder tube to a 15 ml centrifuge tube. The total volume in the centrifuge tube should not exceed 10 ml. The contents should be left in the dark for a few hours to ensure the complete removal of all extractable pigments.

Note (e) During the extraction period, pigments are very photosensitive and neither extracts nor the unextracted filters should be exposed to strong sunlight or else chlorophyll values will be reduced to a small fraction of their initial level in less than an hour. Tubes must be stored in complete darkness.

Note (f) The period of extraction should be about 15-20 hours. After this time, the rate of further extraction is too slow for an extension to be merited. Pigment extracts should preferably be kept chilled; but they can be kept at room temperature for many hours without deterioration. If cells are pretreated in a grinder (see Note d), any further extraction is slow; but for safety, tubes should be stored for a few hours to complete the leaching of cell fragments.

Note (g) The use of 10 ml of solution in a 10 cm path-length cell is recommended for maximum sensitivity. Greater sensitivity can be obtained by using 10 cm cells containing less than 10 ml; but this is scarcely great enough to warrant the increased manipulative difficulties. The ultimate sensitivity is, in practice, more dependent upon the size and reproducibility of blanks. Glass filters disintegrate to pulp, instead of dissolving in acetone, and the pulp retains at least 1 ml of solvent. To ensure enough extract to fill a 10 cm cell, therefore, 12 ml of acetone rather than 10 ml should be used.

Note (h) Centrifugation should be as efficient as possible when Millipore filters are used. Three to four thousand R.P.M. for about 10 minutes is generally satisfactory in most small centrifuges, but the efficiency should be tested with each instrument used. Difficulties may be encountered when centrifuging down the glass pulp from glass filters. Tubes should be centrifuged for 1-2 minutes to pack most of the fibers to the bottom. The centrifuge is then stopped, the tubes removed, and glass fibers adhering to the walls of the tubes above the level of the solvent are taken down into the bulk of the liquid by gently splashing the walls by flicking the tubes. The tubes are then returned to the centrifuge and spun for about five minutes. If this precaution is not taken, some fibers held above the solvent layer may enter the spectrophotometer cell.

Note (i) These extracts should not be allowed to evaporate and should be exposed only to subdued light for the briefest possible period. The measurement of absorbance against acetone (instead of against water) is

recommended as acetone has markedly less absorption in a 10 cm cell at 750 $m\mu$ than has distilled water.

Note (j) The wavelength setting of the spectrophotometer used should be checked against a standard hydrogen or neon line source because the precision of the present method depends upon settings being correct to better than 20-30 $m\mu$. With quartz prisms at wavelengths exceeding 600 very slight movements of the optical system, brought about by vibrations, etc., can easily result in errors of five $m\mu$ or more in wavelength settings. If a suitable lamp is not available, check the extinction of a suitably concentrated plant extract and adjust the spectrophotometer, if necessary, until a maximum extinction is obtained at 663 $m\mu$.

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